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Mathematical Analysis

by
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University of
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JOURNAL OF MORPHOLOGY.

YOLK-NUCLEUS AND POLAR RINGS.

KATHARINE FOOT, EVANSTON, ILL.

POLAR rings have been observed in *Clepsine* by Grube, Leuckart, Robin, and Whitman,¹ in *Rhynchelmis*, by Vejdovsky,² and in *Allolobophora foetida*³ by the author.

In the present paper I hope to prove that the polar rings and the so-called yolk-nucleus are one and the same substance, and that therefore the material which forms these structures is by no means confined to the three forms just mentioned. In the fall of 1894, I identified the granular masses of cytoplasm found in the ovarian egg with the polar rings of later stages, and traced the substance step by step during the growth of the maturing and fertilized egg; but the publication of this work was reserved to form part of a later paper on the maturation and fertilization of the egg of *Allolobophora foetida*.

¹ C. O. Whitman, "The Embryology of *Clepsine*." *Quart. Journ. of Micr. Sci.*, vol. XVIII, 1878, p. 234.

² F. Vejdovsky, "Entwicklungsgeschichtliche Untersuchungen." Prag, 1892.

³ "Preliminary Note on the Maturation and Fertilization of the Egg of *Allolobophora foetida*. JOURNAL OF MORPHOLOGY, vol. IX, 1894.

In the following winter, the ovarian egg of *Lumbricus* was studied by Mr. Calkins¹ in the laboratory of Professor E. B. Wilson of Columbia College. His results differ so radically from those obtained by the study of *Allolobophora foetida* that I think it best to give a brief account of my results. Calkins's results are, briefly, as follows: "The yolk-nucleus is chromatin in the form of granules." "This granular mass disintegrates and the parts form the yolk plates of the egg."

In *Allolobophora foetida* the "yolk-nucleus" can be sharply differentiated from the chromatin, and in normal eggs I find no structures answering to the "great yolk plates" described and figured by Calkins for *Lumbricus*. In a few cases, how-

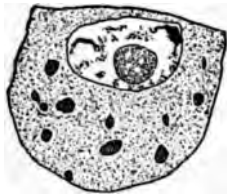


FIG. 1. — Section of a degenerating egg from ovary of *All. foet.* Abbe camera.

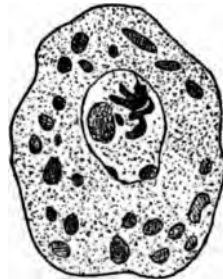


FIG. 2. — Calkins's Fig. 5 reduced one-half.

ever, in ovaries from worms past the breeding season (clitellar region no longer marked), I find, among eggs in various stages of degeneration, some that exactly correspond to Calkins's figure 5; the chromatin has for the most part lost its granular structure, forming one or more solid homogeneous masses among the granules, and in the cytoplasm there are large homogeneous masses, which appear to be identical with Calkins's "great yolk plates."

Ovaries from worms without a defined clitellum show various stages of degeneration. In some cases the eggs of one ovary will be entirely degenerated, while those of the other will appear normal; again, only the older eggs of one or both ovaries will show degeneration; while in some cases both

¹ Gary N. Calkins, "Observations on the Yolk-nucleus in the Egg of *Lumbricus*." *Transactions New York Acad. of Sci.*, June, 1895.

ovaries will appear entirely normal. I am inclined to believe that the condition of the ovary in this respect depends upon the length of time that has elapsed since the ceasing of its functional activity.

METHOD.

The origin and formation of the polar rings were first traced by various methods, none of which, however, differentiated the substance from all other constituents of the cell. The fixatives used were chromo-acetic, corrosive sublimate, corrosive acetic, Hermann's fluid, Hermann's fluid followed by Merkel, Merkel's fluid, osmic acid of various strengths, picro-acetic, Parenyi's, Flemming's, etc. The stains used were various haematoxylin, various carmines, and numerous anilins. While I was engaged in a systematic effort to find a stain that would differentiate the polar-ring substance from other constituents of the cell, the publication of Calkins's results from his study of *Lumbricus* made it still more important for me to substantiate my results by differential staining. The use of lithium carmine and Lyon's blue was suggested to me by seeing some beautiful specimens of nerve tissue, prepared (under the direction of Dr. Patten) by Miss Lewis of Harvard University; and I am indebted to Dr. Patten for subsequent advice as to the use of the stains. Shortly afterwards I found that Korschelt¹ in 1889, had used practically the same method (borax carmine and Lyon's blue) to differentiate the yolk-nucleus (*Dotterkern*) of various insect eggs; and in spite of his beautiful results (clearly differentiating *Dotterkern* from chromatin), I find very few investigators who have repeated his method.²

¹ Korschelt, "Beiträge zur Morphologie und Physiologie des Zellkernes. *Zool. Jahrb.*, Bd. IV, Hft. 1, 1889.

² Calkins seems to have tried Korschelt's method, and he says of it: "After borax carmine and Lyon's blue the yolk-nucleus and chromatin had the bright red stain of the carmine." In *Allolobophora foetida* the only eggs that do not give a constant reaction by this method are eggs in some stages of degeneration; for example, the "great yolk plates" (?) of Cut 1 deeply stain with carmine, and persistently retain the red after treatment with Lyon's blue long enough to stain the degenerating cytoplasm deeply.

The method that has proved most satisfactory for eggs of *Allolobophora foetida* is to stain the sections from one to twenty-four hours in lithium carmine, wash in acidulated alcohol for a few seconds, and double stain with a very dilute solution of Lyon's blue. The length of time required for staining depends upon the fixative used. The process must be carefully watched under the microscope; for the lithium carmine in both the spindle fibres and cytoplasmic network may be replaced by the Lyon's blue.¹ If the staining be properly modified to suit the special fixative, all the fixatives tested give results more or less satisfactory; but corrosive sublimate, with or without acetic acid, gives the most brilliant and satisfactory reaction. A more detailed description of the effect of the various fixatives upon the polar ring substance will be given later.

Before describing the figures, I would emphasize the fact that each is an exact representation of the preparation both in form and color, most of the work having been done by an expert draftsman and colorist (Mr. H. Bridgham); not even in shade or tint is there any variation from the original. None of the figures are in the least diagrammatic; they were drawn from sections, the camera lucida being used in all cases. Each egg (taken from a cocoon) was studied under a Zeiss 2mm. immersion lens, and drawn with the aid of an Abbe camera, before it was imbedded for sectioning.² The variations in size of eggs of nearly the same stages of development are largely due to unequal shrinkage, dependent upon the fixative used.

Fig. 1 represents a very young oöcyte. In the cytoplasm, in close contact with the nucleus and sharply differentiated from the chromatin, nucleolus, and cytoplasm, is a blue, granular mass,—the so-called yolk-nucleus. This substance

¹ If the oögonial areas are overstained with Lyon's blue, the nuclei appear imbedded in a matrix of blue; the cell boundaries, red cytoplasm, and capillaries being obliterated. If preparations fixed by Hermann's fluid are overstained, the nucleoli as well as the yolk-nucleus will stain blue.

² These facts are emphasized in order to prevent a mistake similar to the one made regarding the figures of my preliminary note. The *American Naturalist*, vol. XXIX, January, 1895, speaks of my "diagrammatic figures."

greatly increases as the egg grows, and can be traced (by the sharp contrast of color) step by step during the development, fertilization, and cleavage. It is present at the chief points of activity in the cell: it is in the spindle, it forms the fertilization cone and the archoplasm of both sperm and egg attraction-spheres; and at the pro-nuclear stage a part of it is aggregated at both poles of the egg, forming the structures known as the polar rings. For this blue substance I shall retain the term employed by Boveri in a far more limited sense — the term *archoplasm*.

In no case where the method has been properly applied have I found a cell in the resting stage, — that is, a cell containing nucleus, nucleolus, and cytoplasm, — without being able to recognize at least a trace of the archoplasm, the cell often being so small as to make it impossible to decide whether it belongs to the earlier or to the final generation of oögonia. As growing oöcytes are found close to the stem of the ovary, surrounded by dividing oögonia, the locality of the cell is not a trustworthy indication of the stage to which it belongs.¹

In Fig. 2 we have an older oöcyte, with the archoplasm increased in proportion to the rest of the cell.

¹ In the spermatogonia of *Salamander mac.*, Meves finds masses of granular substance, which he identifies with the "yolk-nucleus" of other authors and with the attraction-sphere. F. Meves, "Ueber eine Metamorphose der Attractionssphäre in den Spermatogonien von *Salamandra maculosa*," pp. 143-4. *Arch. f. mik. Anat.*, Bd. 44, Hft. 1, 1894. Through the courtesy of Dr. Watasé, I have been able to apply Korschelt's method to the sperm cells of *Siren*, and I find that these contain a granular substance similar to the archoplasm of *Allolobophora foetida*. Many of them resemble very closely my Fig. 1 (a young oöcyte). These results recall Nussbaum's identification of the *Nebenkern* of a variety of gland cells with the *Dotterkern* of eggs and the *Nebenkern* of the spermatocytes: "Dagegen wird man den Nebenkern der Drüsenzellen wohl mit dem von Wittich entdeckten Dotterkern der Eier, dem durch von la Valette St. George zuerst bekannt gewordenen Nebenkern der Spermatocyten, den von Leydig aus der Epidermis von Pelobates-Larven beschriebenen Bildungen in eine Kategorie bringen dürfen." Moritz Nussbaum, "Ueber den Bau und die Thätigkeit der Drüsen." *Arch. f. mik. Anat.*, Bd. 21, 1882, pp. 343-4. Later, Balbiani says: "Le noyau vitellin (Dotterkern) des Araneides est l'homologue du Nebenkern (centrosome de Platner) des cellules seminales et du centrosome des cellules somatiques." E. G. Balbiani, "Centrosome et Dotterkern." *Journ. de l'Anat. et de la Physiol.*, tome XXIX, 1893.

Fig. 3 represents a still larger oöcyte, with a corresponding growth of the archoplasm.

Figs. 4 and 5 represent two types of the distribution of the archoplasm found in the older oöcytes. In some cases the greater part of the substance is aggregated at the periphery of the egg; in others the distribution is more nearly equal throughout the cytoplasm.¹

Fig. 6 shows a new phase of the archoplasm.² Here we not only find it distributed through the cytoplasm, but at points where the membrane of the germinal vesicle has disappeared we see it entering into the germinal vesicle itself.

The presence of the archoplasm in the germinal vesicle reminds one of Korschelt's³ and Jordan's⁴ observations on the entrance of the "yolk-nucleus" into the germinal vesicle, and Lavdowsky's⁵ claim to have seen yolk elements within the nucleus. It also recalls the observations of some of the investigators who claim to have seen not only the nucleoli, but also chromatin, *leaving* the germinal vesicle; for this substance in earlier stages has been positively asserted to be chromatin by several authors. In Fig. 6 an attempt has been made to represent the less transparent appearance of the ovarian eggs at the free end of the ovary. A comparison of this with the foregoing figures will show what I have attempted to indicate. With very few exceptions (dependent upon the fixative) this same lack of transparency is shown in all later stages; *i.e.* in eggs taken from the cocoon.

¹ Fig. 4 recalls the *Diffuse Dotterkern* of Stuhlmann: "Die Reifung des Arthropodeneies nach Beobachtungen an Insekten, Spinnen, Myriopoden und Peripatus." Ber. d. Naturf. Ges. z. Freiburg i. Br., Bd. 1, 1886. Cf. His Fig. 137, Taf. VIII.

² The lithographer has not sufficiently blended the three masses of archoplasm at the upper left side of the figure. In the original sketch, the masses are more granular in appearance and are connected by less dense areas of the same substance.

³ Eugen Korschelt, "Beiträge zur Morphologie und Physiologie des Zellkernes." Zool. Jahrb., Bd. IV, Hft. 1, 1889.

⁴ Edwin O. Jordan, "The Habits and Development of the Newt." JOURNAL OF MORPHOLOGY, vol. VIII, 1893.

⁵ M. Lavdowsky, "Von der Entstehung der chromatischen und achromatischen Substanzen in den tierischen und pflanzlichen Zellen." Anatomische Hefte: Merkel und Bonnet's Anat. und Entwicklungsg., Bd. IV, Hft. 13, 1894.

The study of eggs killed in any fixative containing osmic acid inclines me to believe that this lack of transparency is due, at least in part, to material absorbed from the body-cavity of the worm, and later from the albuminous contents of the cocoon; for all these eggs contain in greater or less degree the black spots (*Deutoplasm*) represented in Fig. 14, which can be dissolved out with warm xylol or ether, their fatty nature thus being proved. These, however, are not the only factors; for the degree of transparency shown by the young ovarian egg is not restored by dissolving away the *Deutoplasm*.

The distribution of the archoplasm in all these eggs is modified by the fixative; corrosive sublimate or corrosive acetic are especially favorable for its study, as they cause it to aggregate into masses, thus producing the sharpest contrast between the red and the blue. In chromo-acetic preparations, on the contrary, the distribution of the archoplasm is much more nearly equal throughout the cytoplasm; but *with all fixatives it is aggregated at the centres of activity of the cell*.

I am inclined to think chromo-acetic the most reliable fixative, for the following reasons:

First, it so fixes the eggs that the subsequent treatment with alcohols produces scarcely perceptible shrinkage; as a rule, eggs measured before killing and after mounting give almost the same diameter.

Second, after chromo-acetic all the structures of the cell may be constantly and sharply defined:—the cytoplasmic network; the attraction-sphere, with its centrosome, archoplasm, and rays; the spindle fibres; the chromosomes; the fertilization cone; the sperm, and the sperm granules. Structures that are distorted or obliterated by many other fixatives may be constantly and distinctly defined with chromo-acetic.

I am inclined to believe that the apparently granular structure of the archoplasm is largely, if not wholly, due to the fixatives; certainly the degree of granulation varies with the different fixatives, and at the points of greatest activity of the cell the fusing of the red and blue substances into a structureless homogeneous mass suggests that (at least in some stages) both

substances may be fluid. (The polar rings in the egg of *Clepsine* consist, according to Whitman, of "a transparent fluid substance.") The granular appearance of the archoplasm is most marked at the points of greatest aggregation; that part aggregated at the poles as polar rings appearing more or less granular with all fixatives.

Fig. 7 shows a typical example of the presence of the archoplasm within the spindle and at its poles¹.

In some cases (possibly caused by the action of the fixative) the archoplasm is not equally distributed in the spindle, but is aggregated at its edges and around the chromosomes, leaving areas entirely free from the substance; again, it is aggregated in longitudinal lines, which appear as heavy blue fibres among the red anastomosing spindle fibres. At the poles of the spindle, we see the red cytoplasmic rays of the attraction-spheres and a pronounced aggregation of the archoplasm. This egg (Fig. 7) was killed in Merkel's fluid, which thus far has proved relatively unfavorable for chromosomes and centrosomes.²

Fig. 8 shows a cross-section through the fertilization cone (the structure which appears to be identical with the "cone of attraction" of other animal forms).³ The archoplasm is aggregated around the sperm, and the part in contact with the sperm appears to be blended with the red cytoplasm, forming a homogeneous mass, suggesting a fluid condition of both substances. This figure gives us a typical illustration of the action of corrosive acetic upon the archoplasm, causing it to form into masses, as mentioned above.

¹ This phase of the archoplasm recalls Strasburger's "Kinoplasm"; Fleming's "Zelle." Merkel und Bonnet's *Ergebn. der Anat. u. Entwickl.*, Bd. III, 1893, pp. 66, 104.

² The lithographer has not indicated the faint chromosomes shown in the original sketch. For the shape of the chromosomes of this spindle see my "Preliminary Note on the Maturation and Fertilization of the Egg of *Allolobophora foetida*," *JOURNAL OF MORPHOLOGY*, vol. IX, p. 94.

³ For further details as to the cone and the fertilization of this egg, see my "Preliminary Note on the Maturation and Fertilization of the Egg of *Allolobophora foetida*," *JOURNAL OF MORPHOLOGY*, vol. IX, 1894. The "sperm granules" give a staining reaction similar to the nucleoli; with aurantia and iron haematoxylin, the nucleoli of the pronuclei, the nucleoli outside the first maturation spindle, and the sperm granules, all stain yellow, while the chromosomes and sperm stain blue.

Fig. 9 represents a longitudinal section through one of the fertilization cones, that part of the sperm which is still within the cone being indicated. In this figure we again see the tendency of the archoplasm to accumulate at the centres of activity; it is collected around the entering sperm. *The extent of the cone is dependent upon the length of sperm within the egg*, and the aggregation of the archoplasm (especially after fixatives that destroy radial structure) seems to be the *sole factor* that gives form to the cone. In applying the same method of staining to the spermatozoön at various stages of its development, I have not been able to obtain any reaction indicating the presence of archoplasm; hence it appears that this relatively large mass of archoplasm of the cone has not been brought into the egg by the sperm. The question is here suggested: Is not the archoplasm of the cone identical with the sperm archoplasm of other eggs? Boveri¹ says: "Auf diesem Stadium nun finden wir das Archoplasm als einen dichten kugeligen Hof um das im Centrum des Eies gelegene Spermatozoon (Fig. 10 und 11, Taf. I; Fig. 26, Taf. II). Es stellt sich an den beweisenden Präparaten als eine beträchtliche Ansammlung einer gleichmässig körnigen Substanz dar, die nach aussen ziemlich scharf abgegrenzt ist, während die übrige Zellsubstanz vollkommen homogen erscheint" (p. 65).

Fig. 10. As soon as the first polar body is constricted off we no longer find a fertilization cone; but we find a sperm attraction-sphere with *blue archoplasm* at the point previously occupied by the blue archoplasm at the apex of the cone. At this stage (Fig. 10) the sperm head is contracted into a relatively short, thick rod, with its attraction-sphere occupying a position nearer the attraction-sphere of the lower pole of the spindle than does the rod itself. The structure of the sperm attraction-sphere is identical with that of the egg attraction-sphere described above (Fig. 7). This figure (Fig. 10) suggests that the archoplasm of both sperm and egg attraction-spheres is furnished by the egg alone. In the case of the sperm attraction-sphere we have evidence only that the middle-piece

¹ Th. Boveri, "Zellen-Studien." Hft. 2, Jena, 1888.

of the sperm produces the centre of activity, around which the archoplasm (*already present in the egg*) aggregates.¹ *In the egg of Allolobophora foetida this blue archoplasmic mass is so pronounced that there can be no question as to its individuality; it is as distinct as the cytoplasmic network itself, thus supporting Boveri's assertion of the specific character of archoplasm.*

In this figure (Fig. 10), drawn from preparations fixed in chromo-acetic, we find not only the centrosome but the red anastomosing rays of the attraction-spheres sharply defined.

At the stage represented in Fig. 11 most of the archoplasm (especially in chromo-acetic preparations) is aggregated at the periphery of the egg, only a relatively small amount being present around the very small male and female pronuclei; but in corrosive acetic preparations the archoplasm is not limited so nearly to the periphery, some masses being present throughout the cytoplasm of the egg. This presence of the polar-ring substance on the periphery of the egg, prior to its aggregation at the poles, supports Vejdovsky's² observations on *Rhynchelmis*.

Fig. 12 represents the stage at which both pronuclei are formed, a large part of the archoplasm having aggregated at the two poles of the egg, thus forming the polar rings. In addition to the archoplasm at the poles, a relatively large amount has aggregated around the pronuclei (one of which is shown in the figure); and at the points where the membrane is breaking down the archoplasm is entering into the pronucleus itself. The archoplasm massed around the pronuclei has a different

¹ In the relatively large spermatozoa of *Amphiuma* (which the kindness of Professor Conklin has enabled me to study), I have obtained a reaction to Lyon's blue both in the middle-piece and in the tail. The red cytoplasm and the blue archoplasm are present in both structures, suggesting that the apparent absence of the archoplasm in the spermatozoön of *Allolobophora foetida* may be due to faulty technique. I think, however, there can be no question that the relatively large amount of archoplasm in the cone and attraction-spheres of the egg of *Allolobophora foetida* is merely an aggregation of the archoplasm *already present in the egg*. This conclusion is in accord with the observations of Wheeler on the egg of *Myzostoma*, in which he finds the archoplasm of the cleavage attraction-spheres furnished by the egg alone. Wm. M. Wheeler, "The Behavior of the Centrosome in the Fertilized Egg of *Myzostoma glabrum* Leuckart." JOURNAL OF MORPHOLOGY, vol. X, 1895.

² F. Vejdovsky, "Entwicklungsgeschichtliche Untersuchungen." Tafs. IV und V, Prag, 1892.

shade of blue from that at the poles. It shows (though in a less degree) the blending of the red and blue described under Fig. 8.

In a somewhat later stage (Fig. 13) the membrane of the pronuclei is entirely gone, the chromatin is in the form of loops, and the archoplasm is present around the loops and in the cytoplasm.

Fig. 14 represents one of the polar rings before staining with Lyon's blue. The egg was killed in Hermann's fluid, and the section was stained for twenty-four hours in lithium carmine. We see here how (in Hermann's preparations) lithium carmine stains the cytoplasm, and does not stain the polar ring substance. This section also represents the black spots referred to above (*Deutoplasm*), which subsequently were completely dissolved away by soaking the sections for twenty-four hours in warm xylol.

In studying the literature on the so-called yolk-nucleus I have found that in many cases the substance follows more or less closely a course of development similar to that of the polar ring substance of this egg. It appears first close to the germinal vesicle, increases in amount as the egg grows, and in some forms becomes distributed in granular masses near the periphery of the egg, while in others it appears as numerous clumps or granules throughout the cytoplasm. In many cases the figures show aggregations of the substance which strongly suggest polar rings. I shall, however, quote only those cases where the author specifically states that the substance is finally aggregated at one or both poles of the egg.¹

Stuhlmann's² results from investigations of a variety of insects furnish the strongest evidence that the *Dotterkern* of these animals and the polar rings of *Allolobophora foetida* are

¹ For the latest historical sketches of the "yolk-nucleus," and for the literature on the subject, see L. F. Henneguy, "Le corps vitellin de Balbiani dans l'oeuf des vertébrés," *Journ. de l'anat. et de la physiol.*, ann. 29, 93; and H. Mertens, "Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux," *Archives de Biologie*, tome XIII, 1893.

² Franz Stuhlmann, "Die Reifung des Arthropodeneies nach Beobachtungen an Insekten, Spinnen, Myriopoden und Peripatus." *Ber. d. naturf. Ges. z. Freiburg*. i. Br., Bd. I 1886.

identical structures. Stuhlmann distinguishes two kinds of *Dotterkern*: i.e. "*Diffuse Dotterkerne*," that which is distributed throughout the cytoplasm (cf. his Fig. 137 with my Fig. 4), and "*Eigentlicher Dotterkern*," that which is aggregated at one of the poles. For examples of polar *Dotterkern* see Figs. 164, 165, Taf. IX, and examples on Taf. X.

In a recent paper by J. W. Hubbard¹ on the "yolk-nucleus" in *Cymatogaster*, he clearly traces the "yolk-nucleus" from the germinal vesicle to one pole of the egg; and his figures show an aggregation of these granules fully as pronounced as either of the polar rings of *Allolobophora foetida*.

Leydig's² description of the granules (in the egg of *Argulus*), which aggregate at both poles of the egg, is strikingly suggestive of the polar rings. "In der Substanz des Spongioplasmas erscheinen jetzt auch wirkliche Granula oder dunkelrandige Körnchen, welche sich zu zwei Haufen an beiden Polen des Eies ansammeln" (p. 300).

I am greatly indebted to Professor Wheeler for generously allowing me to study his *Myzostoma* preparations, and providing me with adult *Myzostoma* to prepare with Korschelt's method. The study of these preparations has shown that in various tissue cells the cytoplasm can be differentiated into two distinct substances, one reacting to lithium carmine and the other to Lyon's blue.

It gives me pleasure to express my great obligation to Dr. Whitman for most kind and thorough criticism of my work.

¹ J. W. Hubbard, "The Yolk-nucleus in *Cymatogaster aggregatus* Gibbons." *Proceed. of the Amer. Philos. Society*, vol. XXXIII, No. 144, 1894.

² Franz Leydig, "Beiträge zur Kenntniss des thierischen Eies im unbefruchteten Zustande." *Zool. Jahr., Abth. f. Anat. u. Ontog.*, Bd. III, Hft. 2, 1888.

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EXPLANATION OF PLATE I.

All figures were drawn from sections; in all but three cases the entire figure was drawn from a single section.

Zeiss. hom. immer., 2 mm., 140 ap.

Abbe Camera.

Figs. 1-5, ocular IV.

Figs. 6-12, ocular II.

Figs. 13-14, ocular IV.

Stains, lithium carmine and Lyon's blue.

Archoplasm ("yolk-nucleus," "archoplasm," polar rings), blue.

Chromatin, nucleolus, and cytoplasm, red.

FIG. 1. Very young oöcyte, p. 4.

FIG. 2. Older oöcyte, p. 5.

FIG. 3. Later stage, p. 6.

FIG. 4. Archoplasm dispersed in irregular patches throughout the oöcyte, p. 6.

FIG. 5. Archoplasm peripherally distributed, p. 6.

FIG. 6. Egg detached from free end of ovary (section through germinal vesicle), p. 6.

FIG. 7. Egg taken from freshly-laid cocoon, showing longitudinal section through first maturation spindle, p. 8.

FIG. 8. Fertilized egg. Transverse section through the fertilization cone, p. 8.

FIG. 9. Longitudinal section through fertilization cone, p. 9.

FIG. 10. Egg after formation of first polar body (archoplasm in polar body).

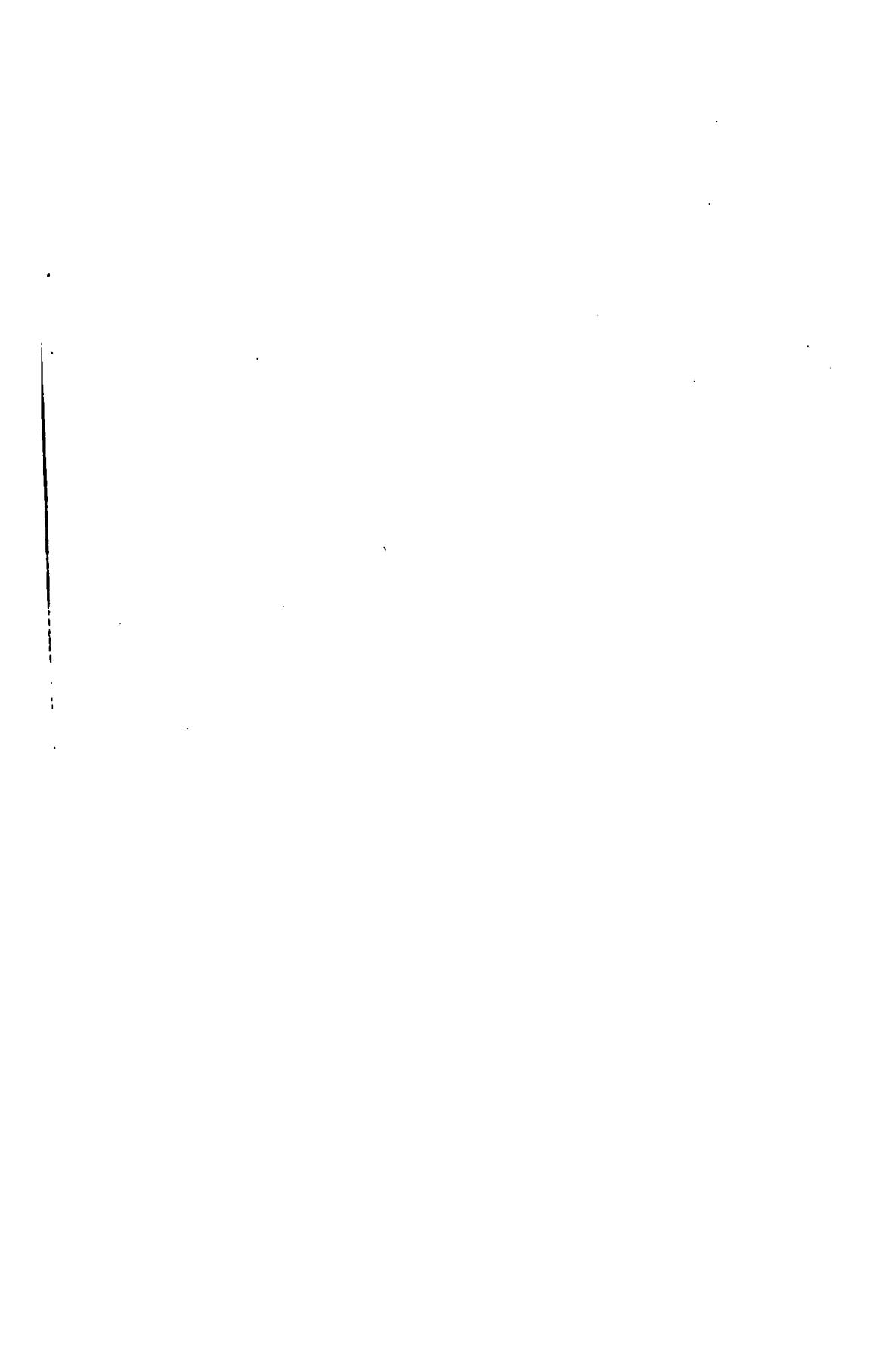
Figure drawn from three sections, p. 9.

FIG. 11. Egg after second polar body has been formed (the polar bodies not represented), p. 10.

FIG. 12. Egg in pronuclear stage; archoplasm aggregated at the poles as polar rings, and concentrated around the pronuclei (only one of which is represented), p. 10.

FIG. 13. Later stage; chromatin in form of loops, p. 11.

FIG. 14. Transverse section of one polar ring, egg in pronuclear stage, p. 11.



VARIATIONS IN THE DEVELOPMENT OF *LIMULUS POLYPHEMUS.*

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(2) There may be an entire absence of certain organs at the outset and a subsequent failure to regenerate the same. This we may assume is due to absence of specific formative material.	
(3) General or local structural weakness of an embryo is indicated by median fusion of its organs in the reverse order of their age and specialization.	
(4) The existence of abnormally large embryos may indicate forced growth or an excess of formative material. Multiple embryos may be explained in the same way. In either case excess of material or excessive growth gives rise to the formation of new organs in reverse order of that in which they disappeared by median fusion and degeneration.	
(5) When the formation of new organs is followed by degeneration, we assume that the multiple parts are formed not from an excess of formative material, but by a kind of forced growth obtained by drawing from a fixed capital.	
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INTRODUCTION.

MOST of the material for the following paper was obtained during the summer of 1891 at the U. S. Fish Commission Laboratory at Woods Holl. The preparations and drawings were made during the following winter. But since then, from time to time, new material was obtained, till the number and variety of abnormal embryos at my disposal became very great. The principal value of the material lies in the large number of abnormal embryos, and in their range of variation, from nearly normal ones to those so modified as to leave a hardly recognizable being behind. To make this range and diversity of variation obvious, great pains were taken to present as large a number of surface views as possible, made from stained and mounted embryos. In nearly every case the embryos were subsequently sectioned, and used as a guide for the interpretation, or correction, if necessary, of the surface

views. But the latter usually tell the whole story, so that but few figures of sections have been deemed necessary.

The amount of good material was so great that I have in no case used embryos for illustration if there was the least doubt of the abnormality being a real one, and not one due to post-mortem abrasion, shrinkage, or other causes of like nature.

The present paper is an unexpected by-product of work along other lines. The time necessary for this digression from a prescribed course was somewhat grudgingly given. It is partly for that reason, and not because their value for this purpose was unappreciated, that I have not entered into a critical discussion of prevalent theories of heredity and development in the light of these new facts, although I have ventured to make a few suggestions of a theoretical character that naturally arose out of their consideration.

It seemed to me that about everything of value in the way of argument has already been said and resaid on the various phases of epigenesis *versus* evolution. When the smoke from the volleys of words discharged in the last few years has cleared away somewhat, it will probably be found that the rival disputants are in closer agreement than has been suspected.

But, after all, the most convincing arguments are the plain solid facts. They are always eloquent, and we cannot have too many. One who has often had occasion to search the literature on a given subject for definite information must be impressed with the uncertainties that hover thickly around the majority of observations, and which render them practically worthless for constructive purposes.

While it is not claimed that the following descriptions are less open to this criticism than most others, it may be a point in their favor that, almost to the last, the embryos were selected and drawn with great care, merely as an illustration of isolated cases of variation, and without any preconceived ideas as to their meaning or mutual relations.

It was my intention originally to publish a description of the normal development first, as I had abundance of material carefully prepared and studied for this purpose. The reader would then be better able to appreciate the meaning of the abnormal

embryos. But in view of the long delay that might attend the publication of the first part, and among other reasons, on account of the interest now centred in variation and abnormal development, it seems advisable not to delay further the publication of the present paper.

But in order to afford some ready means of comparison with normal embryos, prepared by my methods and interpreted on the same basis as the abnormal ones, Pl. I has been introduced, in which are represented the more important stages in the development of normal embryos.

Methods of Hardening, Staining, and Clearing the Embryo for Surface Views and for Sections.

Surface views of opaque embryos are useful for some purposes, certain points being brought out with special clearness shortly after the eggs are put into the hardening fluid. But in order to make out many important details, it is absolutely essential to stain the egg, and clear in clove oil, balsam, or oil of cedar. The latter often gave the best pictures and the eggs could be kept longer in this fluid without discoloring the yolk, than in oil of cloves.

To obtain the best surface views, the embryos should be stained and mounted as soon as possible after hardening, or if that is not convenient, preserved in perfectly clean alcohol in glass-stoppered bottles, as the tannin, or other substances in cork stoppers, is dissolved out by the alcohol and discolors the yolk.

Either picro-nitric or undiluted picro-sulphuric acid or Perenyi's fluid may be used for hardening. The eggs should be immersed in *the cold solution from ten to twenty-four hours*. The yolk is thus made quite hard, and the membranes swell so that they can be easily removed by fine-pointed forceps. The membranes must always be removed before placing in alcohol; otherwise they shrink, and the embryos are injured or distorted. Moreover, a white albuminoid substance collects under the membranes, and is precipitated on the embryo when it is put into alcohol, obscuring very much the beauty and clearness of the preparations.

After the eggs are shelled they are rinsed in the hardening fluids and transferred to a large quantity of strong alcohol, say 94%, which is changed frequently the first few days. If not treated in this way, the yolk is likely to swell and crack. Brittleness of the yolk is much diminished by the prolonged treatment with acids.

The most beautiful surface views are obtained by staining the whole egg in borax carmine, or almost any haematoxylin, for a very short time, one-half to one or two minutes, and then washing in acid alcohol. This method gives very sharp and luminous surface contours. It has been used on a great variety of objects, arthropod and vertebrate embryos, etc., and is very useful.

But this method cannot be used if there is any surface cuticle present. The whole egg must in that case be stained throughout, and the color subsequently drawn completely out of the yolk by acid alcohol (10 to 15 drops strong hydrochloric acid to 100 c.c. of 70% alcohol). The decolorizing process may require several days, and the acid alcohol must be changed as often as it is discolored. When this is successfully done, the yolk has the transparent, yellow color it had before staining, but the nuclei are bright red.

If the eggs are to be mounted, after clearing in oil of cloves, they are split in halves with a delicate knife, made by grinding the end of a needle down to a very thin blade.

The halves with the embryos on them are arranged like serial sections, in shallow cells, and fastened in position with a small drop of thick collodion and clove oil. The fixative is hardened by washing in turpentine, and finally the eggs are mounted in balsam.

Each embryo was numbered, drawn, and in most cases finally taken out of the balsam and sectioned.

The embryos were studied with raised condenser and wide open diaphragm, so that they appeared bright red on a clear yellow field.

In such preparations the elevated surfaces and protruding organs appear dark, the depressions light. All the drawings have been outlined with the aid of a camera from such prepara-

tions, and, with few exceptions, drawn to the same scale, so that the difference in size of embryos of the same age is very evident.

Methods of Obtaining Material.

My attention was first drawn to the presence of abnormal embryos, by finding a nest in which about 90% of the eggs were about ready to hatch, while the remaining ones were apparently in very early stages of development. A few of the latter contained double monsters. A number of different nests were then examined, and many were found containing abnormal eggs. The number of abnormal eggs ranged, at a rough estimate, not by actual count, from none to about 10%, or perhaps more.

In order to obtain a greater abundance and variety of abnormal embryos, about 50,000 eggs, from many different nests and in different stages of development, were collected. The sand containing the eggs was placed in shallow dishes and stirred about by strong currents of water till the eggs rose to the surface, when they were poured off into other jars and washed again till perfectly clean. Placed in hatching jars, they sink to the bottom, and are kept constantly but gently agitated by a current of pure sea water. As fast as the young larvae begin to swim about, they are swept out of the top of the jar through the escape pipe.

After from eight to ten weeks most of the larvae have hatched. *Many thousands of the larvae thus obtained have been examined, and no abnormal embryos found among them.*

In the residue left in the jar are many apparently normal and well advanced embryos, some of which hatch from time to time for many weeks or even six months or more after the regular period of hatching has passed. All those that eventually leave the membranes, whether early or late, and swim about freely, are, with very rare exceptions, normal. Among the remaining eggs are found embryos in all stages of degeneration. A very few only are dead and decomposing. The rest appear fresh and healthy, and one would not suspect from their general outward appearance that they were abnormal embryos.

The eggs left in jars that had been running from ten to fourteen weeks were finally treated with hardening fluids, making the shape of the embryos very conspicuous. The whole collection could then be examined under a dissecting microscope, and the most important forms picked out and shelled at once.

As many embryos were already far advanced (six to nine weeks) when placed in the hatching jar, it is evident that the remaining abnormal forms were at least ten to fourteen weeks old, and some may have been even more than twenty, although as seen by the figures, many of them appear to be not more than from ten days to two weeks old. Most of the monstrosities are apparently the result of retrogressive development, rather than of a slow, progressive one. That is, embryos normal in outward appearance may develop up to stage *E*, Pl. I. They then appear to develop less rapidly, or they may remain for a long time practically stationary. But they finally become smaller, and by the fusion and complete atrophy of their various organs, dwindle to some insignificant remnant which is in turn absorbed. Thus a once well-grown embryo may disappear completely, leaving a healthy-looking egg behind, but one which consists of nothing but yolk.

I was able to confirm the above statement by selecting a dozen or more abnormal embryos and keeping them under observation for four or five weeks. It is difficult to make out details on living eggs, but enough could be seen to prove beyond any doubt that in each case the characteristic abnormality, such as asymmetry, fusion, or reduction of appendages, etc., became more and more marked from day to day, until my observations were brought to a close by the complete disappearance of the embryos, due to the increasing opacity of the chorion. On killing and staining these eggs, the embryos could be easily distinguished again, in advanced stages of degeneration.

In other instances I have kept large numbers of the residual eggs in open dishes, for five or six weeks after they were taken from the hatching jars. In such cases, the number of individuals showing extreme median fusion, or atrophy, or general degeneration, seemed to be greater than before. The number

of abnormal embryos seemed to be greatest in the nests taken from sand that was blackened and foul from decaying organic matter (decomposing eggs?); but on the other hand, they were often found in abundance in perfectly clean sand close to nests where nearly all the eggs were normal.

It occurred to me that the abnormal eggs were produced by the unusual conditions in a hatching jar, such as the constant movement and the exposure to light, or perhaps to the difference in the temperature or density of the water.

This last summer, therefore, hoping to obtain new classes of variation, about 25,000 eggs were placed in shallow dishes. Some were kept in the dark, others in the direct rays of the sun. The water in some dishes was allowed to evaporate almost to dryness, leaving a thick crust of salt in them. A large quantity of fresh water was then added. Under this treatment, many of the older larvae died, and their bodies were allowed to putrefy in the dishes, so that the water became very foul. At the close of the season, all the eggs that survived these indignities, about two-thirds of them, were brought to Hanover, and kept in shallow covered dishes, exposed during some part of the day to the direct rays of the sun. Up to November, the water was turbid and filled with bacteria. Shortly after that, algae began to grow, and now cover the sides and bottom of the dish with a thick, green scum. In December, it was thought a large number of abnormal embryos might be obtained, *and about 5000 were killed and examined, including trilobite larvae and unhatched eggs, but not a single abnormal larva or embryo was found in the lot!* This fact is not easily explained, because in any case we ought to find a certain number of abnormal forms. But it seemed probable that all those embryos originally abnormal were early exterminated by this drastic treatment, and only the normal ones survived, that is, normal in every respect except their very slow development, and this was probably due in part at least to the increased density of the sea water through evaporation.

Causes of Variation.

It is assumed that all the embryos were at first normal in external appearance, and that the condition in which they were found was attained by a gradual fusion and atrophy of organs, according to the methods described under the various headings. But we have always had in mind the possibility, indeed the strong probability, that in some cases the normal form was not actually expressed, but that the embryo from the first appeared in some one of the various stages of fusion and degeneration. For example, when a particular appendage is completely or partially invaginated, we have no means of determining whether it was first normally formed and was subsequently invaginated, or whether at the moment of its first appearance it gradually assumed the condition in which we find it. The one condition may be regarded as the resultant of the simultaneous action of normal and abnormal factors, the other the resultant of the abnormal factors acting after the normal ones have found expression. No number of cases, however great or however varied, can settle that point. But a complete series of them properly arranged may be taken to indicate the successive stages a fully developed normal appendage would assume when subsequently invaginated, and the same applies to any of the other modifications that have been observed.

All variations of the same class are no doubt due to the action of similar abnormal factors that tend to throw the normal mechanism out of its beaten track. But while similar factors will tend to produce similar divergences in the end, the nature of the preceding forms will depend on the intensity of their action and on the period in the development at which they begin to act. The variations first to appear are of the greatest import, because the at first slight divergence becomes continually greater till it leads to impossible combinations which may necessarily be fatal to the future organism. Moreover, an organ on its way toward a position of stability is more readily affected by external agents than one that has settled down as it were into that mature and stable condition characteristic of all older

organs. But there is nothing to indicate with certainty whether the initial cause of variation is due (1) to a variation in the combination, or the quantity, or the quality of the original constructive materials, or (2) to the variation in conditions external to the ovum, or (3) to a combination of both. But as very divergent forms appear among eggs kept under apparently the same conditions, it would seem more than likely that the first set of causes are the real ones.

Variations in the unfertilized ova, and in the spermatozoa or polar globules, at once arise before the mind in their familiar attitude, as factors in some way connected with the phenomena of variation. And there is the whole infinitely complex Weismannian mechanism, with its endless army of corpuscular brownies, on whose sins of omission or commission we may easily throw the responsibility.

But these corpuscular theories fail to explain anything. Their agents come or go at the beck and call of him who commands them. We are in the end left with the sterile formula, that this or that organ is as it is because the necessary corpuscles were there to make it so !

All the variations so far observed can be traced back to variation in the relative rate of growth, specialization, and degeneration in the numerous groups of cells that constitute the embryo. The problem then is to explain why one group of cells grows faster or slower in one embryo than in another, or why it grows at all. But the conditions determining differential growth are very complex, and may be different in each particular case. No simple general statement will suffice. We have passed that stage where it is enough to know that a machine is made of iron and run by electricity. We must know what electricity is, and the structure and bearing of every pin, screw, and wheel in the whole mechanism. It is needless to say that we are very far from having any such knowledge even of the simplest bit of living matter.

The facts here presented enable us to catch a glimpse of embryological processes under new conditions and from a different standpoint, and while many of them increase rather than diminish the existing difficulties, they will perhaps, in

some future theories of development, find their proper place and partial explanation.

It seemed inadvisable to add further to this paper by detailed reference to the voluminous literature bearing only indirectly on the facts here set forth.

So little is known about variations in arthropod embryos, and especially arachnids, and the facts we present are so different in character from those already known, that no injustice will be done previous workers along similar lines by not referring in detail to their publications. The only reference to an abnormality in *Limulus* that I have been able to find is in an article on "Diploteratology, An Essay on Compound Human Monsters," by Geo. J. Fisher, Albany, 1868. A good figure is there given of an adult (?) animal with a double caudal spine and a symmetrically forked abdomen.

DESCRIPTION OF THE DIFFERENT CLASSES OF VARIATION.

I. INVAGINATION OF APPENDAGES.

This remarkable modification is of comparatively common occurrence in forms which are in other respects more or less abnormal. It is confined, so far as I have observed, to the thoracic appendages, and most commonly affects the middle ones of the series.

It may begin after the appendage is fully formed as a minute, slit-like depression at its distal end, Figs. 10, 11, *th. ap.*⁴, *th. ap.*³. The slit is always transverse to the long axis of the body, and appears in the stained specimens as a fine line in the middle of a clear band devoid of nuclei. When the invagination is complete, the whole appendage is carried inward, so that in its place is an opening leading into a deep tube with a flattened, conical lumen.

The third or fourth appendages on either or both sides may be invaginated, Figs. 10, 11, or, as in one case — the only one observed — all the thoracic appendages, with the exception of the first and sixth pairs, may be invaginated, the infolding being

greatest in the second pair, and diminishing in depth from that point backwards.

The embryos just described are in other respects nearly normal, the principal deviations being in the abbreviation and atrophy of the abdominal region in Figs. 8 and 10, and the modification of the cephalic lobes in Figs. 10 and 11. But invaginated appendages are frequently found in other types of embryos, as in Figs. 14, 16, 18, 33, 34, 40, 61, 65, 70, 98.

Any appendage, except perhaps those of the first pair, may be invaginated; the third pair appear to be most frequently affected in this way.

Sections have been cut through several invaginated appendages, and confirm completely the conclusions drawn from surface views. They throw little further light on the subject. Pl. X, Fig. 10, represents a longitudinal, vertical section through the embryo shown in Fig. 10. The only point of further interest here is seen at the inner end of the appendage, where the ectoderm seems to become continuous with the mesoderm, as though there was an inward cell proliferation at that point. If such were the case, a communication, through the hollow appendage, might be established between the exterior on one side, and the mesenteron on the other. A condition would then result like that which obtains in the gill slits of vertebrates.

As to the cause of the invagination, very little can be said further than that it is a local, internal, rather than an external one.

We cannot see how any of the general external conditions, such as the density or the composition of the surrounding medium, could produce such a purely local effect as the invagination of one out of several apparently identical appendages.

There is no evidence whatever that local pressure, such as that which the egg membranes, or the adjacent appendages, might exert, was the cause of the invaginations, for the membranes stand far away from the embryos at this period, and besides, an examination of most any of the figures shows clearly that the position of the invaginated appendages is such that the membranes could not touch them. Such cases as

those shown in Figs. 10, 11, or 65 could not be due to pressure of membranes or of adjacent appendages.

It seems to me, therefore, that the immediate cause is a local, internal one, independent, to the same extent as the normal growths, of external conditions, and having its source in some remote instability of the innermost mechanism.

This class of variations may therefore be called *normal variations*, to distinguish them from those due to the more immediate action of the environment. They must be regarded as necessary incidents of a particular structure and likely to occur in a certain percentage of cases, irrespective of the immediate environment.

In vital processes, what may be at one time or place an incidental and occasional phenomenon, may become elsewhere under other conditions a constantly recurring result. It is therefore clear that in estimating relationships by means of morphological characters, such variations deserve careful consideration. They are as likely to throw light on phyllogenetic problems as ontogeny. The latter indicates the established paths connecting the present with the past; the study of normal variations shows us possible paths leading out of the present into the future.

These facts, then, are interesting morphologically in two ways:

(1) They may be regarded as forming an indirect confirmation of the view that the lung-books of scorpions and spiders, as claimed by Lankester and Kingsley, are invaginated gill-bearing appendages, modified for breathing air.

(2) I have maintained in a former paper on the "Origin of Vertebrates from Arachnids," that the complete visceral arches of vertebrates are homologous with the appendages of an arachnid-like ancestor, because in *Limulus* and scorpions, the number of these appendages, their innervation, the position of their important sense organs, the structure of the meso-blastic cavities associated with them, and the nature of the muscles arising from these cavities, resemble as a whole the corresponding structures in vertebrates.

Aside from other considerations, the striking difference between arthropod appendages and the gill arches of verte-

brates renders any relation between them at first sight very improbable. But the unexpected discovery that in this particular arthropod the appendages are frequently invaginated, leaving in their place a series of slit-like gill openings, such as those shown in Fig. 1, is a potent factor in favor of my view.

If in Fig. 8, the thoracic appendages had been provided with gill leaves like those on the abdominal appendages, we would then have, in place of typical arthropod appendages, a series of respiratory sacs, the cavities of which, by the persistence of embryonic conditions might, after the manner of vertebrate gills, communicate with the alimentary canal, either through its nephridium and somite, or by a secondary opening at the apex, where ectoderm and mesoderm appear to be continuous. The exact relations in *Limulus* of the invaginated appendages to the mesoblastic somite, and of the latter to endoderm have not been determined, as in the cases studied the cavity of the somite had disappeared. But there seems to be no reason to doubt, from what occurs in the normal embryos of *Limulus* and other arthropods, that something like the condition mentioned above might arise, for each of the assumed conditions is known to occur.

The great difficulties involved in determining what is ectodermic, mesodermic, or endodermic in the vertebrate head, we are just beginning to realize. The use of the terms is founded on the supposition that the embryological processes in vertebrates present a modification of those assumed to occur in some real or imaginary invertebrate, which is further assumed to be a more or less remote ancestor of the vertebrates. While the problem is still in this uncertain condition, the fact that these "a priori" methods of interpretation do not harmonize with the suggestions here made cannot be used as an argument against them.

II. ABSENCE OF APPENDAGES.

We shall consider all embryos showing the absence of one or more appendages under the above heading. In all cases the reduction of an appendage seems to be accompanied by a

degeneration more or less complete of all the other organs on the same half metamere, but as these organs are less easily seen, I have confined my statements in the main to the appendages. The degeneration of an appendage is an indication that degeneration has taken place or will take place in the other organs of that half-metamere, but the reverse is not true, for an appendage frequently persists long after all associated organs have disappeared. Absence of appendages is one of the most common abnormalities, and like the invagination of appendages is most likely to occur in embryos showing other indications of abnormality. *The three anterior thoracic, first, second, and third, and the abdominal appendages are most frequently absent.* The three posterior pairs of thoracic appendages are found so frequently after everything else has disappeared, that one might suppose a nauplius-like larva was a normal feature in the development of *Limulus*, but a little consideration will show that such is not the case.

While the appendages are frequently absent in pairs, leaving bilaterally symmetrical embryos, this is by no means the rule.¹

A. Absence of Abdominal Appendages.—This is perhaps the most common of all the modifications observed. A great many of the abnormal embryos showed some abbreviation of the abdomen, accompanied by the reduction or the entire absence of the appendages.

Fig. 5 shows the condition of the abdomen in normal embryos at the time all the thoracic appendages have appeared. The primitive streak is still present as a narrow, longitudinal furrow, from the floor of which there is an inward proliferation of cells.

One frequently finds embryos, otherwise normal, in which the marginal fold, *m.f.*, which represents the margin of the future thoracic and abdominal shields, sweeps across the median line just back of the first pair of thoracic appendages. They are so much like what occurs in Fig. 8, that it was not necessary to represent them. In some of these cases, the

¹ I have unfortunately not tabulated all the cases seen, but I have a strong impression that degeneration is more likely to affect a half metamere than a whole one; as though the former were the unit of structure rather than the latter.

abdomen is perhaps merely retarded and may appear later in its normal condition. This is probably the case with Figs. 9, 10, and 15.

The Telopore. — In the normal embryos, at a very early stage, the posterior end of the body, or the anal plate, consists of a broad mass of proliferating cells, representing a modification of the posterior of the two primitive cumuli that constitute the beginning of the embryo.

In Fig. 1, there is already formed a primitive streak-like invagination in the anal plate, representing a specialization of this proliferating area. Along this proliferating furrow, ectoderm and inner-layer cells become continuous. Without following out its history in further detail here, it is enough to state that it persists, either as a solid mass of cells or as a furrow, up to stage *C*, after which it gradually disappears. The anus does not appear on the flat abdominal plate till about stage *E*, Fig. 7, long after the primitive streak has disappeared. But these normal conditions are frequently deviated from, giving rise to a great range of variations.

In many cases when the abdomen is abbreviated or absent, the entire remaining region, where the abdomen should be formed, is invaginated to form a deep depression, varying greatly in size and general appearance. This depression, or *telopore*, is not a modification of the anus or of the primitive streak, for one or the other of them may, in some cases, be found at the bottom of the depression. It seems to be the result of the active proliferation that is going on in this region during the formation of new segments.

A common form of the telopore is shown in Figs. 9, 10, and 11, another in Figs. 16, 19, 21, 22, 23, 24, 36, 59, 62, 67, 68. In Figs. 20, 21, 23, 51, the whole abdominal region is so deeply depressed that the posterior thoracic and abdominal appendages, when they are developed, are carried into the cavity.

At the bottom of the depression, I have often found in sections the proliferating primitive streak, not markedly different from that in the normal position.

The anus may finally appear at the bottom of the telopore, after the primitive streak has disappeared. In some cases, it

is difficult to distinguish the telopore from the anal invagination, or in other cases from the primitive streak, so great is the range and number of modifications that may be observed.

The infolded abdominal region seems to straighten out in some cases, and in the end give rise to a normally segmented abdomen. In the majority of cases, however, its presence indicates a general weakness that leads ultimately to complete degeneration of the whole embryo. In some cases the defect persists to very late stages, for trilobite larvae are not uncommon that are perfectly formed except for the abdomen, which may show any one of the numerous stages of degeneration.

Whether these defective larvae die, or the abdomen is restored after successive moults, is not known. I have looked in vain for traces of aborted abdomens and in fact for any kind of abnormalities in individuals older than the tribolite stage.

B. Asymmetry of Abdominal Appendages has been observed in a large number of cases. This condition is well shown in Figs. 29 and 31, where there are three abdominal appendages on the right but no trace of them on the left. In Fig. 29 the abdomen is thrown round to the left by the unequal development, and a peculiar, hood-like fold of ectoderm covers its posterior portion.

In Fig. 30, there are four abdominal appendages on the left, and only two on the right. In Figs. 32, 37, 38, 53, 54, there is strongly marked asymmetry.

C. Absence of Thoracic Appendages. In the normal embryos, the second, third, and fourth thoracic appendages appear simultaneously, and shortly afterwards in the order named, the first, fifth, and sixth.

In the abnormal forms, any one thoracic appendage, or almost any combination of the twelve, may be absent, but, as one might infer, from what has been said in reference to the invagination of appendages, it is very difficult to determine in any given case whether the appendages failed to develop, or whether their absence is due to degeneration.

In order to obtain some light on this point, a dozen or more living abnormal embryos were kept under observation from the 20th of July till the 17th of August. Most of the eggs

either died or became so opaque that after a few days nothing was clearly visible in them. It was, however, clearly established, by means of careful drawings made from time to time on such eggs as were transparent enough to allow one to follow the changes going on within them, that a gradual atrophy of the anterior and posterior extremities took place, while the whole embryo showed a marked decrease in size. These facts were enough to show that progressive degeneration did take place in these embryos, and it is very probable that a similar gradual atrophy would have taken place in most of the cases of abnormal embryos here cited, if they had been allowed to live long enough.

The simplest cases of the absence of thoracic appendages are those where one or more appendages are absent on one side of the body, as in Pl. IV, Fig. 30, where the right second and third are absent. Similar cases are shown in Figs. 13, 14, 29, 30, 32, 33, 34, 35, 36, 37, and others.

These cases are common, but such as that in Fig. 38 are extremely rare, only three similar ones having been seen. In this embryo the whole of the left cephalic lobe, nerve-cord, and mesoderm, — everything, in fact, except what appears to be the left sixth appendage, is absent, while on the right side everything is perfectly normal, except for the slight spiral curvature to the left due to the unequal cell stress.

A very frequent form of abnormality is where there has been a *nearly bilaterally symmetrical reduction of the anterior, or the posterior, end of the embryo, or both*. The cases, in so far as they affect the abdomen, have already been considered. This process carried still farther would affect the reduction of the posterior part of the thorax, but that condition seems to be comparatively rare. There is on the contrary a tendency to leave this region intact, reducing instead the three anterior segments one by one, from before backwards, producing conditions like those in Figs. 16, 18, 24, 25, 26, 27. The small embryos with but three pairs of appendages represent the last stages of the process, and they are so abundant that I at one time supposed they might represent a true *nauplius* stage. It may be that similar embryos gave rise to the statements of

Dohrn and Osborn to that effect. But that they are not nauplii is obvious because they are mere fragments of embryos, minus brain, oesophagus, and one or more of the anterior thoracic segments. One cannot always determine with certainty just what appendages are preserved in these false nauplii, but in most cases they appear to be the three posterior pairs of thoracic appendages.

There are three reasons in favor of this view: (1) In some cases that clearly belong to this category, the cephalic lobes and the two or three following segments are quite rudimentary, showing marked anterior degeneration, while the three posterior appendages are of fair size. In Fig. 25 is a typical case. Here the cephalic lobes are reduced to a rounded plate of cells with the remnant of the oesophagus in the centre, and they are separated from the thorax by a wide space along which no organs are developed.

(2) In cases where there has been anterior degeneration, accompanied by the fusion of right and left halves, the last three pairs of appendages (known to be such by the presence of the flabellum on the sixth) undergo the least modification or degeneration, and persist long after every organ in front of them has disappeared. Figs. 44, 48, also 94, 96, 97, 102-104.

(3) When transverse fission occurs, it divides the embryo between the third and fourth pairs of thoracic appendages into two parts; the posterior one usually persists for a long time after the first part has disappeared, showing that it has the greatest vitality. Figs. 51, 98, 103.

It is therefore very probable that in Figs. 25, 26, 27, 33, 34, 35, 62, 65, 66 the segments present are the 4th, 5th, and 6th thoracic. But it must be observed that in Figs. 31 and 32 the second and third pairs of appendages are much better developed than the posterior ones. And also in Fig. 27 the rudiments of the oesophagus and cephalic lobes lie directly in front of the first pair of appendages. The same position of the cephalic lobes and oesophagus is observed in Figs. 33, 34, 45, 62, 65, 66, 67. It is thus obvious that entire segments may be omitted without leaving any apparent break in the series. This is especially clear in Fig. 27, where three thoracic segments are

certainly absent, because the cephalic lobes and abdominal appendages are in their proper relative positions, and yet only three out of the six thoracic segments are present. It cannot therefore be argued that because in Fig. 62 the oesophagus lies just in front of the first pair of remaining appendages, that they are the original first pair and not the fourth.

Figs. 33, 34, 35, 65, 66 probably represent modifications of this three-legged form in which the appendages have undergone various modifications through invagination, median fusion, and degeneration.

In Fig. 34, the last pair have fused to form a median appendage, and in Fig. 35 there has been such distortion and reduction that it is hard to recognize what remains, but it appears to consist of the three left thoracic appendages and one right. The oesophagus lies at *oe.*, and the telepore at *tp.*, at the bottom of a furrow partly covered by a hood-like fold.

In most of these small three-legged forms, the median ventral surface is deeply depressed and closely surrounded by a thick and high marginal fold.

III. MULTIPLICATION OF APPENDAGES.

Multiplication of definite regions or organs of the body, not including in this category double or triple monsters, is very rare. I have observed but one case. It is interesting and important, since it proves conclusively that a half of a metamere already laid down has the power to multiply independently of adjacent organs. This is shown in Pl. II, Fig. 12, where the right chelicera—which fortunately can be identified here with absolute certainty—has divided twice. The first division apparently gave rise to a^{1+2} and a^{3+4} . A subsequent division separated completely a^3 from a^4 . But the third division effected only a partial separation of a^1 from a^2 . The abnormal growth has also modified the right nerve-cord at this point, throwing the head over to the left. None of the neuromeres are very clearly brought out in this preparation, so it is not certain whether there is one for each of the four right *helicerae* or not. But there is a prominent enlargement of the

right nerve-cord, *s*, which from its shape and position appears more intimately related to the third left chelicera than to any of the others. What appears to be the neuromere of the fourth right chelicera is small and triangular, and pushed to one side by the growth of the neuromere just in front of it. That this growth affects the whole right half of the segment is shown by the presence there of two rudimentary lateral eyes, *i.e.*

IV. FUSION OF RIGHT AND LEFT HALVES OF THE EMBRYO AND ANTERO-POSTERIOR DEGENERATION.

This remarkable phenomenon has been observed in so many different stages, that there can be no doubt as to the manner in which it takes place. Median fusion and degeneration begins at the anterior end of the embryo (except in the hour-glass type), and gradually extends toward the posterior end. In the typical cases, each organ in one half of the embryo unites with its fellow of the opposite side to form a median unpaired organ. Those nearest the median line unite first, and then degenerate, and those lateral to them follow in the order of their position, till the whole of the segment has disappeared. The steps in the process are most clearly shown by the appendages, the dorsal organs, and the nerve-cords. The other paired organs probably fuse and degenerate in the same manner, but owing to their indistinctness in surface views, it is not so easy to follow in detail their successive modifications. In this way Δ -shaped embryos are produced, showing various stages in the progress of the degeneration from the anterior toward the posterior end.

Toward the close of the process we may find either a median row of papillae, representing two or three pairs of medianly fused appendages, Pl. VI, Fig 50, or an exhausted mass of cells at what was the posterior end of the embryo; and finally these useless remnants may in their turn disappear. The principle involved in the process is well illustrated by the adjacent Figs. 1 and 2.

In Fig. 1, each square represents a segmental organ of some kind. The lower half of the diagram shows how the organs

are produced by the usual method of apical growth, or rather it is more correctly a double rectilinear growth, — a longitudinal one and a lateral one at right angles to it. The longitudinal growth may be represented by the lines parallel with $A-p$, and the lateral growth by the lines parallel with $E-E$. The relative age of each organ is determined by its position in relation to these two sets of lines, the uppermost a being the oldest and most specialized organ in the body, and the lowest A the youngest. Now it is obvious on examining the diagrams that the median fusion and antero-posterior degeneration of the organs takes place in the reverse order of their formation, the oldest dying first and the youngest last. Considering for the present only the small-lettered part of Fig. 1, median fusion and degeneration such as occurs in *Limulus* will gradually carry the hypothenuses of the shaded triangles toward each other till they meet in the median line, the shaded areas themselves gradually disappearing. The half of the embryo

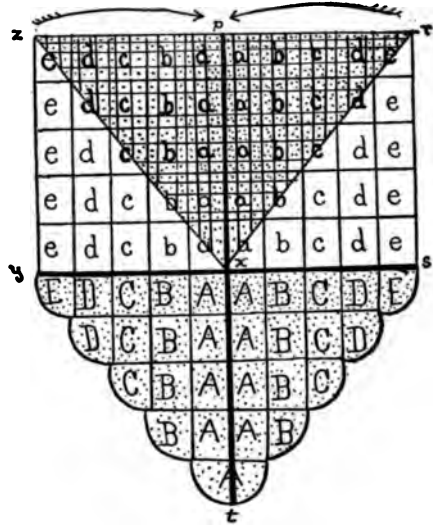


FIG. 1.

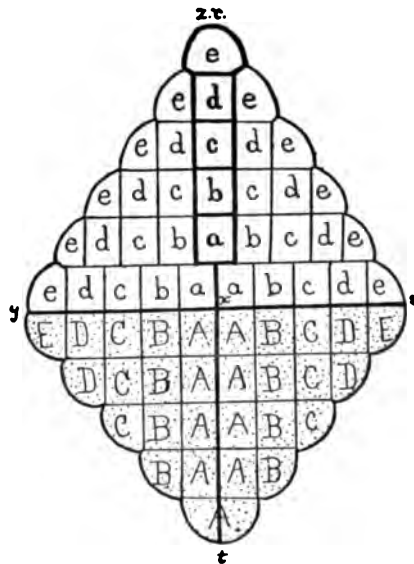


FIG. 2.

Diagrams to illustrate the laws of growth of segmental organs and their disappearance by median fusion.

that dies first, therefore, consists of the two quarters *s.p.x.* and *r.p.x.*, and with their disappearance the remaining quarters are thrown toward the median line in such a way that the position of each organ is shifted a step farther toward the median line than the corresponding organ just behind it. Furthermore a row of dissimilar, unpaired organs is formed along the median line in the same sequence from behind forwards, as from the median line toward the sides, Fig. 2, *a, b, c, d, e*, and *A, B, C, D, E*.

The steps in this process are as follows. The most median organs, *a* and *a* in the anterior row, Fig. 1, fuse with each other in the median line and then disappear. Their place is immediately occupied by the fused organs that were originally next to them on the outer side, namely *b* and *b*; at the same time *a* and *a* fuse in the second row. In the third step, the fused organs *b* and *b* of the first row disappear and the fused organs *c* and *c* take their place; *b* and *b* take the place of *a+a* in the second row, and *a* and *a* fuse in the third. The following steps are of the same nature and are repeated till the condition like that in the anterior part of Fig. 2 is reached. It may be continued still farther till all the organs have disappeared, the last fused organs to disappear being the two most lateral ones of the first line.¹

In reality, however, no such condition as the one just described would ever be completely realized, owing to the presence at the posterior growing end of the body of groups of segments in which the organs are arranged in an inverse manner to that just described, see lower half of Fig. 2. The tendency will be, therefore, to produce a body in which the arrangement of the organs is determined by the interaction of growth and degeneration, one having its greatest activity at the posterior, the other at the anterior, end. The resultant form will be therefore a more or less elongated rhomboid. The tendency of all segmented animals to assume such a form,

¹ It is not claimed that the process of median fusion and degeneration always follows exactly the steps indicated above, as will be observed on examination of the surface views of various embryos, but the variations from it are not of such a nature as to invalidate the general law.

owing to the fusion or reduction of organs at the extremities of the body, and the fullness and completeness of structure shown in the intermediate regions, testify to the universality of this double law of growth and degeneration.

What is the *cause* of this mode of degeneration? It seems to be an exaggeration of the forces which have determined the form and manner of growth in normal embryos.

Assume, for example, that the embryo of a segmented animal consists of a double row of independent half-metameres, placed with their oldest ends or *heads* toward each other, and growing laterally like an acrogenous plant. The series of organs thus formed, extending from the oldest or median end to the youngest, or lateral, extremity, such as the endoderm,¹ mesoderm, neuromere, nephridium, appendage sense-organs, and various somatic structures, will then appear on the half-somite, roughly speaking, in the order of the appearance of such organs throughout the animal series. And they are analogous to the metameres of a whole animal, or to the succession of morphological units produced in an acrogenous plant. There is, however, a sharp distinction to be drawn between the arrangement of organs on a half-metamere and the succession of metameres in a segmented animal.

In the latter case we have a succession of homologous parts more or less modified by their position; in the former, a succession of fundamentally different parts — a logical sequence of morphological structures in accordance with the genesis of physiological specialization. The dorsal and ventral surfaces are thus forever fixed as parts fundamentally different, and less likely to be confounded through secondary changes than the anterior and posterior extremities of the body. Broadly speaking, therefore, the ventral surface of a segmented animal is the oldest and most specialized, — the dorsal the youngest and least specialized.

If we had to deal only with lateral growth, — *i.e.* growth at the apex of each half-metamere, and with the formation of new half-metameres at the posterior end, and all this taking place on

¹ The endodermic and mesodermic portions of a half-metamere are enfolded at an early period, and consequently do not appear to form a part of the series.

a *flat*, instead of a spherical surface, — we should have a succession of organs like that in the lower half of Figs. 1 and 2. But each half-metamere not only grows in length but in width,

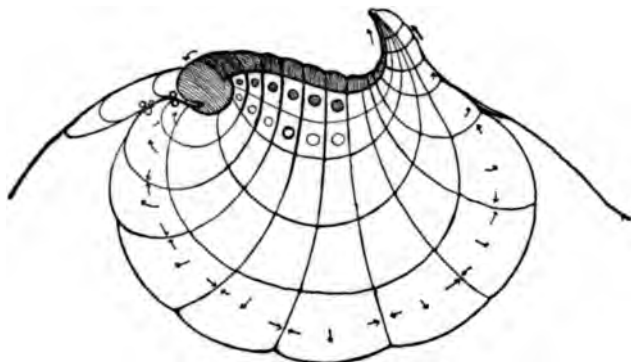


FIG. 3.

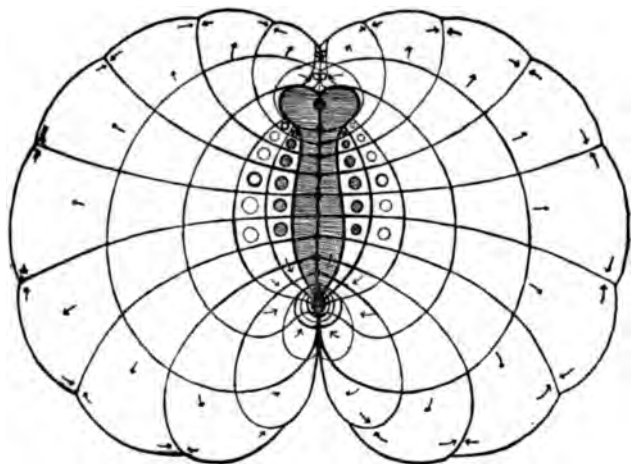


FIG. 4.

Diagrams to illustrate how a double series of half-metameres formed in succession from before backwards, and growing on a spherical surface, will produce differential growth forces. These in turn produce median concrescence and degeneration, cervical and caudal flexures, and the modification of the trunk into regions of different morphological and physiological potentialities.

and as this is most manifest at the lateral end, they will tend to be triangular in form. Moreover, each succeeding metamere will have to grow under other conditions of cell tension and available yolk surface than the metamere that preceded it.

The whole tendency will be then to produce a form like that in Fig. 4, where an attempt has been made to illustrate the action of growth forces by lines corresponding with the arrangement of organs. The increase in width of the lateral ends of the metameres will produce a constantly increasing tension which will find relief, and thus favor still further growth, by movements forwards and backwards. The ends of the most anterior metameres will thus be forced together along the median line, causing the median fusion of organs in front of the true anterior extremity of the body. Conditions like these have probably caused the fusion of such organs as the median ocelli and the olfactory organs of *Limulus*, and in insects the upper lip, which arises, as has been repeatedly shown, from the fusion of originally paired organs lying on the very anterior margin of the cephalic lobes. (See "Eyes of *Acilius*.")

At the posterior end of the body, concrescence of the mesodermic area is the result, and the true apex of the body is shut off from growth over the surface of the yolk. The new segments formed after this period are therefore forced to grow vertically upward and forwards. Hence the conical, forwardly directed tail seen so constantly in vertebrates and arthropods.

The segments formed at the apex of this conical tail lobe will be produced under conditions very different from those found elsewhere, and we can readily see how these conditions might not only be the direct cause of the diminution in size and fusion of the organs there, but also prohibit the further addition of new segments.

At the head end there is no necessity for such a form, because no new segments are formed there. But there is a gradual thickening of all the organs along the median line, which tends to find relief forwards as well as laterally ; but as the anterior margin of the cephalic lobes is, as it were, shut out from the median line by the ingrowth of the mesodermic area, its only relief is in buckling upward and forwards. Hence the cerebral flexure, and the general S-shaped contour of the whole embryo, Fig. 3.

Concrescence of the margins of the mesodermic area occurs in the normal embryos, as shown by the figures in Pl. I.

But there are some very interesting phases of it seen in the embryos that are much reduced in size, and which may be best considered in this connection. The margin of the mesodermic area is there frequently enlarged so that it becomes very conspicuous in surface views. It serves as a pretty safe index of the grade of development and degeneration, and also to identify the posterior end of the body in embryos reduced to such low terms as those in Pls. VI and VII. This structure is formed by the fusion of the peripheral margin of the mesoblastic somites with the ectoderm and with the yolk cells.¹ *A long primitive, streak-like thickening is thus formed, by the proliferation of which the germ layers are extended laterally in exactly the same way that we are familiar with as occurring at the posterior end of the body.*

There is a great difference in the lateral extension of the segmented portion of the mesoderm. In normal embryos it may reach the very margin of the mesodermic area, while in abnormal cases it may not extend beyond the appendages. But there is universal agreement in normal and abnormal embryos in the union at the periphery of ectoderm, mesoderm, and yolk cells.

The lateral growth then of a metamere is comparable with the posterior apical growth by which the body of the embryo is increased in length.

In normal embryos, the lateral margins of the mesoblastic segments, when first formed, are quite indistinct, Fig. 1, but they soon fuse to form the conspicuous marginal thickening just described, Figs. 2-4, *m.a.* The egg being nearly spherical, the anterior and posterior portions of the margin have a shorter distance to go in order to unite with each other in the median line than the middle portions. For this reason and others that we have already discussed, the margins, following the path of least resistance, tend to form anterior and posterior loops, which finally meet in front of and behind the embryo, Pl. I, Fig. 5, forming a rounded, mesodermic area in the centre of which the

¹ We cannot for lack of space discuss the origin and history of the mesoderm here in the detailed manner that it deserves. We can only point out briefly the more important facts that bear on the subject matter of this paper.

embryo lies. Its thickened rim, *m. a.*, usually forms two conspicuous posteriorly directed loops, which may be found in all stages of concrescence behind the apex of the abdomen. The sides of the loops concresce like the closing of the arms of a Δ , and a longitudinal post-anal thickening is thus formed, below which lies a great cloud of cells, brought together at that point by the union of the proliferating rim.

In the abnormal and degenerate forms, the margin of the mesodermic area is usually very distinct, although every outward trace of segmentation in the mesoderm, and even the greater part of the embryo itself, may have disappeared.

The margin also shows in many cases more clearly than in the normal embryos the posterior, median concrescence, and it takes place in such a manner as to bring very forcibly to mind the similar phenomena in vertebrate embryos. One of the most striking instances of this exaggeration of the mesodermic margin is shown in Pl. VI, Fig. 63. Unlike all the rest, this is an opaque embryo shown by reflected light. The margin of the mesodermic area forms a conspicuous ridge, which is thickened posteriorly. Where concrescence has taken place, a broad median elevation is produced.

Nothing like the thickened margin here described is known to occur in any other arthropod, but a similar concrescence of mesodermic segments probably occurs in all arthropods.

In the embryos very far advanced in degeneration, the margin of the mesodermic area breaks up into isolated, irregular masses containing closely packed nuclei, Pls. VI and VII, Figs. 69, 72, 76, 88, etc. These masses may lie deeply in the yolk, but they still show very clearly their derivation. They frequently send out pseudopodia-like streamers of nuclei, which fade at the distal ends as though they were gradually dissolving in the yolk.

In Figs. 67 and 72, the margins of the mesodermic area first united a short distance behind the end of the abdomen, leaving a pear-shaped area of yolk, covered by the blastoderm only.

In Figs. 10, 18, 24, 61, 68, the margins have fused over a long distance, leaving along the line of fusion a cloud of yolk and mesoderm cells. The posterior margin of the fused areas

is usually distinctly indented in such cases, as though the concrescence were proceeding still farther backwards.

Various modifications of the different phases of concrescence are shown in Figs. 14, 16, 18, 20, 24, 43, 49, 59, 61, 62, 63, 65, 67, 68, 70, 71, 72, 79, 80, 82, 83.

The traces of segmentation in the mesodermic area and the very conspicuous concrescing folds are interesting features of Figs. 14 and 20. Observe also the curious knob-like ectodermic thickening at the posterior end of the concresced area in Fig. 16, a not infrequent occurrence. Compare also Pl. III, Fig. 20 with Pl. V, Fig. 49, where the heart is apparently formed for a part of its length.

The whole process of concrescence, as shown by these embryos, reminds one of the concrescence of the so-called margin of the blastopore in vertebrates. The principal difference is that in vertebrates the ectoderm, mesoderm, and endoderm grow over the yolk together, there being no single layered blastoderm covering the whole yolk before the germ layers begin to form, as is the case with *Limulus* and the arthropods generally. But the presence of this layer of cells can hardly be regarded as a serious objection to a comparison of the processes of concrescence in these two great groups of animals.

In the light of this comparison, the fact that in very rare cases, as shown by Ryder in certain fish eggs, the segmentation of the mesoderm extends to the margin of the concrescing lips of the "blastopore" is very significant. It seems to me most easily explained as a reversion to a condition like that in arthropods.

There are some other interesting suggestions that arise from this comparison. We must not forget that *in Limulus at any rate the growth backwards of this line of concrescence does not increase the body in length. It is merely a part of the haemal surface, thrown back temporarily upon the neural surface by the presence of the yolk. The true increase in length of the axis of the body comes from the proliferation of cells in a primitive streak that lies just in front of where concrescence began.* It is obvious that an application of these principles of growth to vertebrate embryos might clear away some of the difficulties

that have arisen in attempting to explain, first, the increase in length at the posterior end, and second, the relation of the primitive streak to the constricted lips of the "blastopore." In fact, the whole manner of interpreting the formation of germ layers in vertebrates may be advantageously reconsidered from this new standpoint.

I can only touch on these points here, but I shall discuss them more fully elsewhere. The figures are too suggestive to be passed over without comment.

That a half-metamere is to be regarded as the unit of structure of segmented animals is shown, above all (1), as already indicated, by their method of growth and direction of specialization, and (2), by the manner in which half-metameres are omitted or increased or diminished in size, rather than whole ones. On this supposition, certain imperfections in the segments of otherwise typically segmented animals can be explained; such, for example, as *spiral segments*, which may be accounted for as due in part to the imperfect union of twin half-metameres on the dorsal side of the body. Instead of meeting each other squarely, and thus forming a line of equalization of forces, they have grown past each other in opposite directions, and thus each forms a more or less perfect spiral!

Polarity and embryonic axes thus appear in a new light. In any segmented animal the embryonic half-metameres group themselves in such a way that they may be represented by axes meeting at a shifting point, as shown in Fig. 5. The line *H. T.* represents the median line and the axis of longitudinal growth, or of *repetition*, and the triangle *abT*, the form that would result if the individual half-metameres did not mutually modify each other.

W.x. and *p.x.* represent the axes of specialization, along which new organs are produced in each half segment. The growth along these axes is at right angles to the median line, and also parallel with it. The tendency to a concentration of organs at the forward end of the embryo, or toward a median fusion and antero-posterior degeneration there, may be explained

as due to the fact that there is greatest tension along those lines, *Hx.*, and less growth power to resist it, because the tissues at those points are oldest, and have already exhausted more of the inherent powers of growth than at *T*.

It may not be venturing too far to claim for this principle still further applicability ; for example, in explaining the more profound and remote physiological differences between the anterior, middle, and posterior regions of the body. Or rather, it would be better to say that this morphological law is the formal

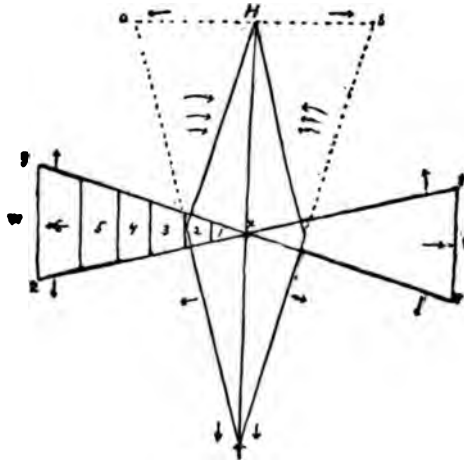


FIG. 5.

expression of the fact that differential growth tension has fixed the posterior, middle, and anterior regions of the body as the seats, respectively, of constructive, elaborative, and destructive physiological processes. But it will not do to press this thought too far, certainly not without a precise statement of the way it is to be applied.

It would also be important to determine whether there is any relation between these laws of growth and decline and the different powers of regeneration shown by various regions of the body, and in this connection we would recall the difference so manifest in this respect between the head and tail region. If such were, indeed, the case, there might be some foundation for the supposition that growth and regeneration are associated

phenomena, antithetical to the associated phenomena of degeneration, specialization, and lack of regenerative power.

We have thus seen how differences in the time and place of growth will in normal embryos produce conditions that cause the fusion and degeneration of organs.

In such cases the fusion and degeneration took place in front of or behind the true extremities of the body. But we see no reason why the same kind of factors should not produce the more extensive median fusion and degeneration seen in the abnormal forms.

This supposition becomes all the more plausible when we consider that the lines of fusion and degeneration are coincident with the lines of greatest stress.

Again, we can see how reversing the conditions that have brought about median fusion and degeneration, *i.e.* diminished lateral cell stress at the anterior end, might permit the formation of double and triple monsters, — as shown by Fig. 7, p. 73.

And finally, if the half-metameres were very much reduced in numbers, the tendency to increase in width at the lateral end would have greater freedom, and more or less ovoid bodies would result, in which the segmentation would be lost sight of in the antero-posterior expansion of the half-metameres. The organs would then tend to be formed along concentric circles, somewhat as in the embryo of a cephalopod.

Having considered what we have regarded as the general principles involved in this class of variations, we will now examine the individual illustrations of the same.

An early stage of fusion is well shown in Pl. V, Fig. 39. The cephalic lobes are constricted, and without character. The chelicerae are absent. The next two appendages are brought closely together, but the space between the appendages of the following pairs becomes greater, till in the last thoracic metamere they are separated from each other by the normal distance.

A tendency in the same direction is seen in Fig. 47, as is shown by the mere trace of cephalic lobes, the absence of

the oesophagus and first two pairs of appendages, and the drawing together of the appendages of the fourth pair.

In Fig. 46 is a more typical condition. The cephalic lobes are entirely absent, and the anterior limbs of the thoracic margin are converging to form the apex of an inverted V. The chelicerae have united at *ch.*, and the second pair of appendages forms a single, long, coiled and slender process, *ap*². The third pair has almost fused, but each appendage still retains its characteristic shape.

In Fig. 44 the second and third pairs have united, while every trace of the cephalic lobes and of the chelicerae has disappeared. In Fig. 42 about the same change has taken place, except that the degeneration of fused appendages has progressed farther backwards, for here both the chelicerae and second pair of appendages have disappeared. The fusion of the appendages of the fourth pair is not quite completed.

Fusion accompanied by degeneration of the two anterior pairs of appendages is shown in Figs. 43 and 49. In Fig. 48 fusion and antero-posterior atrophy have given rise to a form frequently seen in which little of the embryo but the three posterior thoracic appendages is left. In Fig. 41 is a similar embryo in which the oesophagus is still visible a long distance in front of the embryo. It is a pit-like depression merging gradually into a long cloud of cells lying below the surface, and extending backward toward the embryo, *d.oe.* These cells represent either the degenerating remnant of the anterior portion of the embryo, or of the oesophagus.

If the process of fusion and degeneration progresses in the way the various stages just described indicate, we should obtain embryos in which all the paired organs have been fused and subsequently absorbed, except the last pair of appendages, or perhaps the tip of the tail. This appears to be the case with the one in Pl. IX, Fig. 106. We should not forget that this embryo has been developing as long as the others here described; and that it really is in a late stage is shown by its size, and by the concrescence of the posterior limbs of the germinal area back of the median appendage or lobe. There is no trace whatever of a nervous system, unless the dark area

around the stomodaeum represents the remnants of the cephalic lobes.

What appears to be a still more degenerate condition is shown in Pl. VI, Fig. 69. Here the inner layer cells of the margin of the germinal area are breaking up into dense, irregular masses of degenerating cells, while every trace of the head is absent.

Degeneration of fused organs does not always proceed in that way, as is illustrated by the remarkable embryo shown in Pl. VI, Fig. 50. This is the only instance observed in which three successive metameres show the same degree of fusion. There is no indication as to what appendages are represented in this embryo, probably the last three thoracic, as we have seen that they have greater vitality than any other organs of the body.

Similar conditions are shown in Figs. 52-58, but complicated by partial transverse fission. See Section VI.

The only exceptions observed to the law of fusion and degeneration illustrated by the preceding figures—to which many more might have been added—are shown in Pl. V, Figs. 40 and 45. In the first, the cephalic lobes and cheliceral segments have disappeared. The second and sixth pairs of appendages are fused, so that the whole embryo is spindle-shaped. The identity of the appendages is determined by the “dorsal organs,” which are very plainly developed. This embryo, then, seems to furnish a case of median fusion and degeneration at both ends. In Fig. 45 the last pair of legs has fused. It probably represents the fifth pair, the sixth having disappeared.

Nearly all of the older multiple embryos show in one or more of the component individuals the typical antero-posterior fusion and degeneration. But a modification of this process occurs there of extreme interest. In such cases the fusion is greatest between the third and fourth thoracic metameres, and diminishes from that point both forward and backward, producing what I have called *hour-glass embryos*. The constriction thus produced may separate the anterior and posterior regions completely. This condition is very beautifully shown

in Pl. VIII, Fig. 98, also in Pl. IX, Figs. 102 and 103. See also pages 67-71.

The ordinary antero-posterior fusion is well shown in Fig. 94, where the left-hand embryo consists merely of a tail, the normal fifth and sixth, and the fused fourth, pairs of thoracic appendages. A mere trace of the anterior part of the embryo is shown at *ae.*, and the well-developed approximated dorsal organs are clearly seen at *d.o.* No nervous system or other organs are visible. In the upper embryo of Fig. 97 fusion and complete degeneration of the anterior end of the embryo are shown. The fourth pair has fused and is much reduced, the fifth is fused and very long and much folded, the sixth pair is approximated, but not fused. In the opposite embryo the process has gone on a little farther, resulting in the fusion of the fifth and sixth pairs. The fourth pair was very small and partly concealed under the folds of the long and much crumpled fifth pair. The dorsal organs in the upper embryo are close together, in the lower they are completely fused, *d.o.*

Still another condition, but similar to that in Fig. 97, is shown in the right-hand embryo of Fig. 96. There is no marginal fold here or any dorsal organ. The tail has been thrown outwards, making a sharp bend in the longitudinal axis, just in front of the sixth pair of legs. The latter is nearly fused; the fourth and fifth pairs completely so. I can form no plausible conjecture as to what the process to the left of the left flabellum may represent. It is entirely out of place as an appendage, so far as we may judge from the other cases. It is important to observe that in Fig. 96, and in both embryos in Fig. 97, the flabella are larger than usual in embryos of this age, resembling, except in position, normal thoracic appendages. The problematical appendage of Fig. 96 may be an extra flabellum belonging to the left fifth thoracic appendage. But no indication of such an organ has ever been seen in any other specimen.

In the curiously aborted embryo seen in Fig. 100, the posterior three or four (?) pairs of thoracic, and two or three abdominal, appendages are fused along the median line. Here, all three thoracic appendages show about the same increase in

length and irregularity in shape, which is very unusual, indicating that they fused nearly simultaneously and to an equal degree.

In Fig. 93 the left-hand embryo, at its anterior end, is medianly fused and partially degenerated. The minute pit *oe.* probably represents the remnant of the fused second pair of appendages.

In Fig. 95 fusion and degeneration result in the formation of a slipper-shaped embryo in which nothing is left but a single median appendage and a pit that may represent the last of an appendage, or perhaps the fused dorsal organs.

In Fig. 98 is represented an extremely interesting condition, due in part to transverse constriction or fission. The constriction, followed by fusion, and finally by degeneration, takes place between the third and fourth pairs of appendages, and diminishes from that point in both directions. In Fig. 103, embryo *A*, the same process is carried a little farther, all the anterior appendages being fused to form a row of four median projections which preserve approximately the relative proportions of the three pairs of appendages from which they arose. In the same figure, fusion and degeneration are carried further in embryo *B*, and further yet in embryo *C*, where nothing is left but the fused dorsal organ at *c*, and the last two fused thoracic appendages.

In the triple embryo shown in Fig. 104, all three individuals are in essentially the same condition as embryo *C* of Fig. 103.

In Fig. 102 we have a triple monster in which embryo *A* is nearly normal. *B* has undergone transverse fission, as in Fig. 90, and the anterior half has undergone degeneration till nothing is left but a single median projection representing an imperfectly fused pair of appendages. Embryo *C* is represented by the fused sixth pair of legs and by traces of the abdomen.

It is an interesting fact that in nearly all cases when there has been undoubted antero-posterior fusion and degeneration, the abdomen with its appendages is nearly normal and very well preserved. This is somewhat surprising, as the abdomen is usually much abbreviated and often entirely absent in embryos that show a tendency to abnormality in other respects than in multiple fission or in median fusion.

It should also be observed that in Figs. 102 and 103, much less clearly in Fig. 104, the embryos, beginning with the most perfect embryo, *A*, show increased concrescence and degeneration as we pass in a spiral to embryos *B* and *C*. This interesting fact will be discussed under the head of double and triple monsters.

Change in Shape of Fused and Degenerating Appendages.

When two appendages unite, they fuse at the base first, and the fusion extends from that point toward the apex. The resultant appendage is at first much longer and more slender than the unfused ones. It is also usually folded back and forth several times, and otherwise irregular in shape. It subsequently diminishes in length, and finally forms a minute, conical papilla arising from the centre of a saucer-shaped depression. The papilla then disappears, leaving a shallow depression that cannot be readily distinguished from the oesophagus or the fused dorsal organs.

As a general rule the order in which fusion takes place is indicated by the length and coiling of the appendages. When there are several fused appendages visible, they show a gradual diminution in these characters from before backwards, as in Figs. 40, 44, 46, and 102. In rare cases the same characters are presented by all the fused appendages of a series as in Figs. 50 and 100.

V. GENERAL PROGRESSIVE DEGENERATION.

A careful study and comparison of many abnormal embryos of various ages and conditions indicates pretty clearly that most of the abnormalities are due to either a local or a general lack of formative energy. We picture to ourselves two sets of factors at work, the action of one being to increase the quantity and diversification of protoplasm, the other to reduce it to its lowest terms. The action of the first may temporarily prevail, but in the end the second factors are certain to prevent the work of the creative ones.

We assume that there are certain conditions resident in the ovum which, under the action of normal surroundings, guide it through a long series of changes to the expression of that form and mode of action characteristic of what we call the normal organism.

Among the embryos of *Limulus*, there are some in which there appears to be a very slow discharge of vital processes, producing the retarded or belated forms ; again, nearly perfect individuals are produced within the normal period, but very much reduced in size throughout, suggesting the small but perfect embryos of *Amphioxus* that have developed from fragments of segmenting ova. There are embryos constituting a third class, in which a particular charge of formative energy was apparently omitted, resulting in the absence of an eye, a leg, a neuromere, or a large, definitely circumscribed area of the embryo, but without visibly affecting the remaining organs. Finally, there is a fourth class, where the embryo seems properly loaded and the various charges properly connected, and it starts off well, following its normal line of flight for a while. But through some inherent defects, the nature of which we cannot even conjecture, progressive development ceases at a point very far from the mark. Then follows a decline, manifested outwardly by a general decrease in size, by fusion and complete atrophy of one organ after the other, till the whole embryo disappears. But as the animal still lives during this decline, and in all outward appearances is sound and healthy, showing even in the last stages the presence of karyokinetic figures, it is obvious that we can only explain this condition by assuming that the death rate among the cells is greater than the birth rate. And as the last survivors are nothing but indifferent, lymphoid cells, we must also assume that the gradual approximation of the death to the birth period cuts off more and more from the period necessary for cell specialization. *The result is a new kind of death for highly organized animals, — one, namely, in which the component cells gradually decrease in number and in specialization till nothing remains of a once complex organism but a few indifferent cells, which in turn themselves disappear by a continuation of the same processes.*

Almost any organ of the body, but more especially the brain, oesophagus, nerve-cord, abdomen, or appendages, may be absent from the start, or may be behind time, or developing normally may quickly disappear, without in any case, so far as could be learned, either affecting neighboring organs or being affected themselves by conditions other than those produced by their own growth.

The variation can be usually traced back to variations in half-metameres. This would seem to indicate that the trunk of the embryo is a group of integral parts arranged in a double series like two rows of segmented animals placed head to head. Each half-metamere seems to be endowed at the outset with a fixed capital of formative material, which when absent in whole or in part, or exhausted, cannot be restored. In addition to this the growth of a half-metamere may be hindered or favored by local mechanical conditions similar to those producing median concrescence and degeneration.

No other supposition, it seems to me, can explain why one leg, for example, out of twelve utterly fails to develop, while the rest go on as usual, although all are equally surrounded by nourishing yolk and by the same medium.

With these considerations in mind we can understand how a weakening in the developmental forces might be indicated by the four following classes of variation, namely: (1) slowness of development, (2) small size, (3) absence of organs here and there, and (4) gradual reduction of the whole body till it completely disappears.

Let us now consider these four classes in more detail.

(I) *Almost every one of the embryos we have figured is behind time*, as we have already explained in stating the methods by which the material was obtained, and we have also described how single organs, such as the appendages, disappear or fail to develop, and that the absence of any organ is often followed by the complete degeneration of the rest of the metamere.

(II) One of the most characteristic features of the following embryos *is their small size*, a fact well brought out by comparing the figures on Plates I, VI, and VII, all of which are drawn to the same scale. In these cases the reduction in size

is permanent, and if very marked seems to lead to the final disappearance of the embryo.

(III) *Absence of individual organs.*

A. Atrophy of the thorax. The most frequent defect in the thorax is the absence of the entire cheliceral segment, a fact of striking significance in connection with its diminutive size throughout the entire group of arachnids.

Further degeneration may do away, one at a time, with the succeeding thoracic segments in their order from before backwards, but usually leaving two, or more frequently, the three posterior ones, nearly intact. This mode of degeneration may not be preceded by the median fusion previously described. The missing organs are either absent from the start or else degenerate very early. Meantime the cephalic lobes and oesophagus may persist, but in variously modified conditions, as shown in Figs. 26, 27, 33, 34, 35, 62, 67. *These facts show that the anterior part of the thorax is the weakest part and most likely to be absent or to degenerate, and that this weakness gradually diminishes towards the posterior end.*

B. When the cephalic lobes show partial degeneration, the reduction seems to take place first along their anterior margins, and to progress backwards independently of anterior degeneration in the thorax. The law of degeneration of the cephalic lobes as here stated is not so clearly shown as in the case of the thorax, on account of the difficulty of distinguishing the parts. But it seems to hold good in Figs. 18, 20, 21, 22, 27, 47. It is perfectly certain, however, that *the cephalic lobes and oesophagus may be preserved after every trace of one or more segments behind them has disappeared*, as in Fig. 47 and in Fig. 25.

The area where degeneration of the anterior margin of the cephalic lobes has taken place, and which has not been covered by the contracting marginal fold, is nearly always flattened and depressed, as shown in Figs. 10, 11, 16, 20, 27, etc.

C. In the abdomen, the same law of degeneration seems to hold good, i.e. *the most anterior abdominal appendages and neuromeres are the first to degenerate, the posterior ones being the most persistent.* It should be borne in mind that the normal growth of the abdominal appendages is similar to that of the

thoracic. For example, three pairs of thoracic appendages, the second, third, and fourth, appear first, followed by a very small pair in front, the chelicerae, and by two pairs of appendages behind; the most precocious part is therefore the middle portion. In the abdomen, the second and third pairs first appear, followed by the rudimentary chelaria in front, and by the remaining appendages behind. Compare Figs. 4, 6, and 7.

In the degenerate embryos shown in Figs. 10 and 15, the only abdominal appendages present appear to be the second and third pairs. It is hard to say whether the ones that should develop back of them are merely belated or have degenerated, the general impression at first sight being that there has been a shortening of the abdomen by suppression of its posterior end. However, in more typical cases, the facts seem to support a different conclusion. For example, a large part of the abdomen may be absent, and in its place may be seen either a conspicuous tail-like projection resembling the post-abdomen in young scorpion embryos, or an ingrowth into the yolk, which varies greatly in size and depth in different individuals. If we may use the presence of this depression as an indication of the position of the original posterior end of the abdomen, *it is obvious that it is the anterior metameres that have disappeared, because we usually find this depression or projection, as the case may be, carried forward to a point just back of the thoracic appendages.* Compare Figs. 8, 13, 16, 20, 21, 24, etc.

In Figs. 29, 30, and 32 the same law is illustrated by a different class of cases. Here, also, we see that the principal loss of material is at the anterior end of the right or the left side of the abdomen, not at its posterior end.

The abdomen, therefore, like an independent embryo, develops its metameres in a sequence similar to that in the thorax. They degenerate from before backwards, and independently of degeneration elsewhere in the embryo.

The cases we have just described show that there are three separate points at which backward degeneration may begin, namely, at the anterior end of the cephalic lobes, at the anterior

end of the thorax, and at the anterior end of the abdomen. Each of the regions between these points may degenerate independently of the other, and in the same way that the whole embryo sometimes does, suggesting the idea that the embryo consists of a chain of imperfect individuals, such as we see in annelids as the result of imperfect fission.

A less strongly marked division occurs in *Limulus*, across the middle of the thorax between the third and fourth segments. It is shown (1) by the marked tendency of the first three segments to degenerate and of the last three to persist, (2) by the transverse fission that occurs there, see following paragraphs (p. 67), and (3) by the presence of an enlarged pair of sense-organs ("dorsal organs") opposite the first of the last three segments, just as the lateral eyes lie opposite the first one of the first three segments.

When the degeneration is incomplete, it may manifest itself by a partial median fusion and a decrease in size of the organs along the lines separating the regions above indicated.

These cross-lines, where degeneration is most likely to occur in Limulus embryos, are the regions showing the least "vitality"; they correspond to the regions where a reduction in size and a tendency to undergo median concrescence are frequently seen in the adult individuals from various groups of arthropods. In Limulus these lines of weakness are cleavage planes separating regions having different potentialities, and corresponding to regions possessing different morphological characters in many other arthropods. Broadly speaking, the salient morphological characters seen in the various regions of the body of arthropods are similar to those that appear as abnormal variations in Limulus embryos; and, as we have seen, these characters are mainly those due to difference in size, specialization, and median fusion, and these in turn have to be referred back primarily to differences in the power of growth.

The four regions of the body in *Limulus* embryos are roughly shown in the adjacent Fig. 6. The lateral constrictions and the transverse lines indicate approximately the position and amount of median fusion and degeneration.

The repetition of three segments in successive regions of

the body is a striking fact, and it should also be observed that each individual part of the embryo, and especially the ends, tends to assume the shape of a single or double V, characteristic of entire embryos undergoing atrophy by median or apical fusion and degeneration.

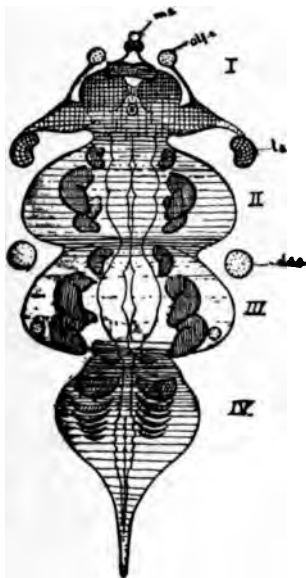


FIG. 6.

Diagram to show lines and regions of most frequent degeneration. The lateral constrictions indicate potential fusion planes due to partial median fusion. The cross-lines indicate the relative weakness of the different regions.

In comparing these variations in *Limulus*, Fig. 6, with the normal conditions in other arthropods, I will merely bring to mind the following facts, to which others might be added.

(a) In the first region we have to recall the absence, or median fusion, or degeneration, of appendages at the anterior margin of the cephalic lobes, especially in insect embryos, and the absence or fusion or degeneration of the anterior pairs of ocelli in arachnid embryos (scorpions, spiders, *Limulus*) and the great development of the antennae and lateral eyes arising from the posterior margins, as in insects and crustacea.¹

(b) The reduction and the frequent median fusion of the appendages just back of the mouth in insects, crustacea and arachnids, and the much greater size of those at the posterior end of the thorax. The frequent absence of the chelicerae in *Limulus* is to be compared with the small size of these appendages in the vast majority of arachnids. The manifest weakness of the first three pairs of thoracic appendages in

¹ The degree of median fusion may therefore be taken as an index of the original order of serially homologous organs. Applied to vertebrates, this would indicate that the most anterior sense-organ is the pineal eye, followed by the olfactory organ and the lateral eyes, just as is the case in *Limulus*. This harmonizes with the conclusions that I have reached on anatomical and embryological grounds of an entirely different nature.

Limulus, as shown by their more frequent absence and median fusion, is comparable with the small size and frequent median fusion of the first three pairs of post-oral appendages in insects and other arthropods.

(c) There is at the anterior end of the abdomen a region of condensation, specialization, and degeneration, forming what I have called the *vagus region* of scorpions and Limulus, and which, in a paper on "The Origin of Vertebrates from Arachnids," I showed was probably of wide distribution in the arachnida, including the trilobites and related forms. In scorpions, it consists of four very much condensed metameres, and in Limulus of two or more, which in both forms are provided with rudimentary appendages, nearly or quite fused.

(d) Finally, the very wide distribution of well-developed terminal appendages through all groups of arthropods is a manifestation of the same law of growth.

In conclusion, therefore, it would appear that the division of the body of arthropods into successive regions composed of several segments is not due primarily to specialization or adaptation, either by or for any particular use, or through disuse, but to some form determining forces that govern growth. The same factors in all probability produce in a similar way metameric segmentation, transverse fission in annelids, and determine the length of the body in a given individual.

(IV) *The complete degeneration of the whole embryo* is an obscure process, and probably varies considerably in different individuals. A continuation of the various local degenerations previously described has, without doubt, gradually produced the misjointed fragments of embryos seen in Pls. VI and VII. They admirably illustrate Empedoclian fancies. The embryos themselves give some indication of the various ways in which they have degenerated. They also indicate that the final stages of degeneration lead to a tolerably uniform condition.

With the disappearance of all the appendages, the embryo may, in the class of cases we shall now consider, be reduced to a mere pit or sac, yet preserving certain features which show

clearly the stage in which the whole embryo would have been, had no degeneration taken place.

Perhaps the best illustration of this is shown in Pl. VII, Fig. 82. The mesodermic area is relatively large, and its posterior margins are thickened and well advanced toward concrescence. There is a very large, projecting tail lobe, like that in Figs. 48, 49, and 94, and *yet of the body proper (which should have all the organs seen in Figs. 5 and 6) there is nothing left but a deep, thick-walled pit, with a triangular opening to the exterior.*

A similar condition is shown in Fig. 83, where the undifferentiated remnant of the body forms a Y-shaped sac with an oval opening at its posterior end.

Figs. 84, 85, 86, 87, and 88 are various modifications of the same condition.

In Fig. 88 the thickened margin of the mesodermic area has broken up into the star-shaped masses of degenerating cells, so frequently seen in the later stages of degeneration.

These embryos represent what is left after invagination, median fusion, and progressive degeneration have attacked with varying success every part of the body.

Most of the embryos shown on Pls. V and VI would probably have reached this condition ultimately. In some of these cases one can distinguish here and there an appendage, or some other organ; but the remaining parts may be so distorted or misplaced that it is impossible to distinguish literally as well as figuratively any head or tail to them, as in Figs. 71 and 56, and others which we have not space to figure.

In Fig. 81 the large posterior depression appears to be the remnant of the anal plate, and the three obscure pits in front of it, the last of three fused pairs of invaginated appendages. In Fig. 80 everything has disappeared except the large pit, situated at what appears to have been the anterior end of the embryo.

Whether the huge projection in Fig. 69, Pl. VI, and Fig. 106, Pl. IX, represents a fused and partly invaginated pair of thoracic appendages or a tail-like projection of the anal plate is hard to determine.

The curious pits and sacs just described themselves finally degenerate into a mere cloud of cells varying in form and appearance, Fig. 89. Many embryos of this kind show slight indications of a central depression, probably the last trace of a sac like those just described.

I have found some eggs among those that had been kept alive for three or four months in which no trace of cells or nuclei was visible in surface views, yet they appeared perfectly sound and free from decay, even when stained and cleared in clove oil. They are probably eggs that have passed so far beyond the stages of degeneration shown in Fig. 89, that even the last few cells have disappeared.

There is another series of degenerated embryos that are very interesting from their resemblance to the early stages of normal embryos. They consist of two groups of cells like two primitive cumuli, one corresponding to the head and the other to the tail end of the body. This condition has already been passed by the embryo shown in Fig. 1.

Whether or no degenerating embryos as a rule pass through this condition with separate *Anlagen* for head and tail is doubtful, nevertheless it is a variety very frequently seen. It is well shown in Figs. 72-78.

There is no way to determine certainly whether an embryo like that in Fig. 73 has degenerated, or whether it has been kept, by something hindering normal development, in approximately its original condition. But the great age of the embryo, the evidence in other embryos like this one, of concrescence, and the breaking up of the margin of the circular mesodermic area, bears out the assumption that it has undergone profound degeneration. The anterior mass of cells and the pit usually seen in its centre probably represent the degenerated cephalic lobes and oesophagus, and the posterior cloud, the anal plate, and telopore. Both of these discs are in all this class of cases about the same, but the posterior one is usually the larger and thicker. Sections of the discs show various conditions, from one where there is merely a thick, homogeneous mass of cells, flush with the surface, but sending into the yolk pseudopodia-like masses of degen-

erating cells, to one in which it consists of a thin layer of cells deeply invaginated in the centre, and from the invaginated part alone arises a cloud of scattered yolk nuclei. Pl. X, Fig. 73.

Whether the depressions in Figs. 72, 79, and 81 represent the oral and anal depressions, or the pits left after the degeneration of fused and invaginated appendages, or of one or more segments, as in Pl. VI, Fig. 61, cannot be determined.

In some cases, as in Figs. 69 and 73, where the outline of the axis of the embryo may be faintly distinguished, there is no cephalic cloud of cells visible.

It would thus appear that degeneration may carry old embryos back to a state resembling that seen in very young embryos, i.e. one where it consists of a cluster of proliferating cells at either end.

These facts seem to indicate that the body of the embryo is not a single organic unit, such as it would be if it were an elongated gastrula with fused lips, but rather one of a double origin.

We may regard the head *Anlage*, from which arise the cephalic lobes and oesophagus, as representing the remnants of a trochosphere, and the posterior *Anlage* as the primitive trunk which arises from the trochosphere as a bud-like outgrowth. I have already shown elsewhere,¹ that the origin of the stomodaeal nerves in *Limulus* supports such a view.

VI. FISSION.

All the cases of fission that I have seen in *Limulus* affected embryos in which the thoracic appendages were well developed, or if they were not present it was evident that their absence was due to degeneration.

The multiple embryos must necessarily, from the methods of obtaining them, have been well advanced; and any abnormality in the early stages is easily overlooked among the hundreds of eggs that one must examine in order to find them. Nevertheless it seems probable that fission does not usually begin till a

¹ Morphology and Physiology of the Brain and Sense-Organs of *Limulus*.

comparatively late period. This is certainly the case with the embryos shown in Figs. 90 and 91, the type most frequently seen.

In the formation of multiple embryos, we may distinguish two kinds of fission:

(1) Transverse fission, dividing the embryo into anterior and posterior portions, the plane of fission being usually between the third and fourth thoracic appendages.

(2) Longitudinal fission, beginning at the anterior end. This is the most common form, and one presenting a great number of modifications through degeneration. *The process is essentially different from that of transverse fission, for the latter is the result of a local transverse concrescence and degeneration, while longitudinal fission consists in the formation of two new halves of an embryo along the median line of one already existing. The formation of the new halves begins at the anterior end and extends gradually backwards, one new half being a mirror image of the other. The old halves are thus thrust apart, and with the newly formed halves make new embryos.*¹

This process may be repeated a second time in one of the new embryos, thus producing three embryos, tail to tail, consisting of the two original halves plus four new ones.

A. TRANSVERSE FISSION.

There are more or less clear indications of this form of fission in a great many embryos, but it is rarely that it is very clearly marked. The fact that the plane of fission occurs at a definite point is very remarkable, and indicates a break in the morphological continuity of the embryo of considerable theoretical interest.

In normal embryos, neither the order of development of the appendages, nor their size or shape, gives any indication of this cleavage line. The great development of the lateral, segmental

¹ Indications of longitudinal fission, beginning at the posterior end and extending forwards, are very rare. No *bona fide* case has been observed, and I doubt whether it ever occurs in *Limulus*, the cases in which it appears to exist being perhaps better explained as malformations of the posterior end of the body, rather than as the beginning of true fission.

sense-organ, *d.o.*, is the first thing to suggest a break in the apparently homogeneous series of thoracic metamerer.

But we frequently see in embryos which in most respects do not depart from the normal type, quite constant differences in the arrangement of the thoracic appendages, which may be regarded as indications of the cleavage plane in question. In Pl. III, Fig. 23, *the potential fissure plane is indicated by an increased distance separating the third and fourth pairs of appendages.* This would be of little importance perhaps taken alone, but such cases are frequently seen, so that it probably has the significance attached to it.

Another class of cases illustrating the same thing consists of embryos in which the *three anterior pairs of appendages have a markedly different direction of growth* from the posterior ones, suggesting the characteristic difference in appearance between the mandibles and maxillae of an insect embryo and the thoracic appendages. These cases are also comparatively common, typical cases being shown in Pl. III, Figs. 19 and 22.

None of these cases would attract attention if it were not for the fact that the constriction about to be described occurs between these two sets of thoracic metamerer.

A most beautiful example of transverse fission, and one that throws a good deal of light on the ones about to be considered, is shown in Pl. IV, Fig. 29. This case is, however, complicated by the partial degeneration of the left half of the thorax, and the complete absence of the left half of the abdomen. The constriction is due to the presence of a transverse line of degeneration having its greatest intensity along the fourth segment.

In Pl. VI, Fig. 51, is an obvious constriction between the third and fourth thoracic metamerer. When the constriction is more conspicuous, it has evidently been preceded by fusion of the right and left halves, the degree of fusion diminishing from the point of greatest constriction toward the anterior and the posterior end. This is beautifully shown in the lower embryo of Pl. VIII, Fig. 98. As every pair of appendages is present in this case, there is no question about the exact point of constriction.

A similar case is shown in the single embryo in Fig. 52. A comparison of this embryo with the preceding leaves little doubt that the constriction has taken place between the third and fourth segments. The cephalic lobes are much degenerated, and the chelicerae are absent. The modification of the remaining appendages is easily determined from the lettering.

In Figs. 54 and 55 are two other illustrations of transverse fission accompanied by degeneration. In Fig. 55 the anterior portion of the thorax, consisting of three pairs of appendages, but without cephalic lobes, oesophagus, or neuromeres, is widely separated from the posterior portion, which consists solely of a conical projection representing either an enlarged caudal lobe, or else the last pair of fused, thoracic appendages. One or two small pits in front of it indicate, probably, where the other posterior thoracic appendages have disappeared. In Fig. 54 is a curious modification not easily explained. It appears to be due to a transverse constriction separating the thorax, the only part that is left, into two portions. The two pairs of appendages of the anterior part have fused to form a median row, the fusion being indicated by the great length of the two twisted median appendages. The appendages were apparently absent on the right side of the posterior portion, so that it is spirally twisted.

In Fig. 53 an earlier stage of the same process is shown. There has been a partial constriction between the third and fourth pairs of thoracic appendages. In the anterior portion the third pair have fused, the second are still widely separated, while the first pair and most of the cephalic lobes are absent. In the posterior portion the fourth pair are fused, and the right fifth and sixth appendages, including their neuromeres, are absent. The fifth and sixth appendages of the left side are very large, and owing to the unequal bilateral development, thrown spirally toward the right. The distortion of the axial line is also shown by the position of the telopore, *t.p.*

Still other embryos where median fusion has played a conspicuous part, but which still show evidences of transverse fission, are shown in Figs. 56, 57, and 58. In Fig. 58 the anterior projection probably represents the remnant of the fused

anterior thoracic appendages; and the posterior infolding, the invaginated remnants of the posterior thoracic appendages. It is not improbable that Figs. 74 to 78, Pl. VII, represent still further degeneration of this kind.

We cannot be certain whether a common form of embryo such as that in Figs. 48 and 94 is due to gradual antero-posterior fusion and degeneration up to the fourth thoracic appendages, or whether there has been previous fission at that point, followed by the degeneration of the anterior portion. But in either case it shows a distinct difference in the vitality of the two parts, which confirms our view of their morphological difference.

In Fig. 103 we have the most remarkable example of constriction, followed by antero-posterior degeneration, that has been met with. The least modified embryo, *A*, is a further modification of the condition seen in the lower embryo in Fig. 98. All the organs in the anterior portion have fused leaving a median row of three long appendages, and fairly developed cephalic lobes and oesophagus.

In embryo *B* the whole anterior portion has degenerated, while the closely approximated dorsal organs appear like a pair of eyes in front of what is left of the posterior part of the thorax.

In embryo *C* the same process is carried still further, the dorsal organs now forming a median pit in front of the remainder of the thorax, analogous to the median eyes of the cephalic lobes.

This condition has been reached by all three of the embryos shown in Fig. 104.

The persistence of the posterior part of the thorax, following transverse fission, is also well shown in the very advanced embryos in Figs. 96, 97, and 100. The point where transverse constriction has occurred, and the degree of antero-posterior degeneration are about the same in each.

That in cases like those just referred to there may be actual separation of the anterior part of the thorax from the posterior is shown by Fig. 102, in embryo *B*, where in the two widely separated portions of the thorax there is nothing left in the anterior one except an imperfectly fused pair of appendages.

In conclusion, therefore, it may be stated that transverse fission occurs in the region of the fourth thoracic segment, dividing the body into two parts, which show different morphological characters and different degrees of vitality. The separation of the two parts is the result of a constriction brought about by the successive median fusion and degeneration of the organs lying along that segment, the most median, and therefore the oldest and most specialized, fusing and degenerating first, and the others following in the order of their position on the segment. There is a gradual diminution of the effects of degeneration both in front of and behind this line. A similar line of degeneration is seen in the region of the cheliceral segment, just back of the cephalic lobes, and in the region of the first abdominal or cheliceral segment, Fig. 6. Further evidence of degeneration at these points is seen in normal adult animals, in the small size of the organs on these segments, and in their tendency to unite in the median line. In the abnormal embryos it is shown by an exaggeration of these conditions, resulting very frequently in the absence of entire segments.

The inherent tendency to diminution of growth along such lines is what has, in all probability, led to the division of the body of arthropods into successive regions, such as cephalic lobes, prothorax, thorax, abdomen, post-abdomen, etc.

B. MEDIAN LONGITUDINAL FISSION; DOUBLE AND TRIPLE EMBRYOS.

This method of forming multiple embryos is comparatively common. It often begins at a late period, after the full number of normal appendages and metameres is formed.

The first steps in the process have not been observed, but a study of Fig. 90, where fission has not progressed very far, shows what the initiatory processes must have been like. There can be no doubt that we have actual fission here, and not fusion of two originally independent embryos. It is also clearly shown by such cases as that in Fig. 98, where the left half of the larger embryo is defective, owing to the lack of the formative material necessary to produce an entire new half; and especially by the fact that in all these cases the embryos

match each other exactly, and always in the same way, which could hardly be the case if two separate embryos had united with each other through accidental contact.

In Figs. 90 and 91, then, if we have fission instead of fusion, one half of each new embryo must come, not from the fission of already existing organs, *but by the formation of two new halves.* Where does this new material come from, and by what processes of growth are the new halves formed?

In answer to the first question we may say at once that there is not the slightest evidence of the existence of any formative material in the shape of proliferating cells along the median line where the new parts are forming. The old halves are, to all appearance, separate from the new. As they are already specialized, and sharply circumscribed, there seems no way open to explain the origin of the new half of a segment by lateral budding, or by regeneration, or by growth, from the corresponding old one. We might suppose, perhaps, that the new neuromeres in Figs. 90 and 91 come from a kind of regeneration of the old one, *but that could not possibly be the case with any of the new organs lateral to the neuromere*, such as the appendages, sense-organs, and margin of the mesodermic area.

We are left, then, entirely to conjecture as to the origin and causation of the new growth. We shall return to this point later.

The new halves are formed, however, in a very definite manner, which we shall now proceed to explain. *In brief, they appear in exactly the reverse order of that by which the old ones disappear by median fusion!*

I. DOUBLE EMBRYOS.

In the formation of double embryos, two new halves belonging ultimately to separate embryos, are produced, each half being the mirror image of the other, to which it is united along its *lateral* margin. The new halves first appear at the anterior end of the median line of the old embryo, probably between the anterior median margins of the cephalic

lobes. They form a triangular body which grows backwards at the apex, and laterally in either direction, along lines parallel with the base of the triangle. The two old halves are thus wedged apart till, with the complete formation of the new halves, the embryos form a straight line, tail to tail. Fig. 92.

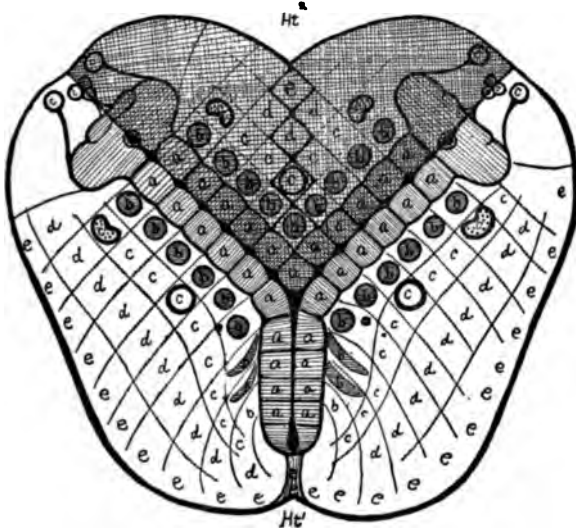


FIG. 7.

Diagram to illustrate the law of formation of new halves in double embryos.
The new halves are shaded.

Each new organ of a metamere appears first as a single organ common to both embryos, and having a normal position for each, Fig. 7. Additional organs are formed in the same way, in the order of their arrangement on the metamere. For example, the organ nearest the median line is formed first; this then divides into two, and the one lateral to it appears between them as a single organ common to both embryos; this divides, and the next one appears in the same place, till all the organs of a given metamere are formed. The same process takes place in the next posterior metamere, but it is always one step behind that in the metamere in front of it.

The result is that in an embryo that has nearly completed its division, as in the diagram (Fig. 7), we find a row of

median, unpaired organs, which in their serial arrangement follow the same order as in the metamere itself, namely, *a*, *b*, *c*, *d*, *e*.

It is thus obvious that the rate and direction of growth is such that each new half tends to form a right-angled triangle, the apex of which coincides with the posterior end of the embryo, the altitude with the mid-ventral line, and the base with the width of the oldest half-metamere.

We thus see a gradually increasing series of new organs appear along the altitude of the triangle, or the old mid-ventral line. They attain their full size and perfection of form for that stage; then each divides into two (the one a mirror image of the other), which move away from the median line, and in their former place appears a new unpaired set, composed of the organs that normally lie lateral to the ones just formed.

The successive eruption of new series of organs along this median line, and the manner in which they divide and move away from it to right and left, is so entirely different from what we have been accustomed to see that it is very impressive. This effect is not diminished on further reflection.

Examination of the diagrammatic figure illustrating an incomplete double embryo shows that each metamere has a lateral growth similar to that which occurs at the posterior end of segmented animals. *In posterior, apical growth a number of like parts, or segments, increasing in age and in differentiation toward the anterior end, is produced. In the lateral growth of a half-metamere a series of unlike parts is produced. But while there is a similar method of growth in both cases, there is in the second case a greater increase of specialization in passing from the growing point toward the part first formed.*

If we carry the process seen in Pl. VIII, Fig. 90, back to its beginning, we are led to conclude that fission began by the formation of new organs in the median line at the very anterior end of the body, that is, in the indentation separating the right and left semicircular lobes of the brain. The first organs to appear then must have been the cephalic lobes. But each half of a cephalic lobe consists of at least two parts, a lateral one, the optic ganglion, and a median one, the semicircular lobe and

the cerebral hemisphere. It is not probable, therefore, that a common median cephalic lobe developed in the same way as a common appendage, for the optic ganglion of one side would have to take the place of the cerebral hemisphere of the other, or *vice versa*. The different parts are probably formed as independent organs in a sequence from the median line laterally, as is the case with the different organs on a metamere. That is, a common semicircular lobe is first formed, then the cerebral hemisphere, then the optic ganglia, and finally the lateral eyes.

In the earliest stage of a double embryo observed, Fig. 90, we see how the characteristic median row of unpaired organs is forming. The fourth neuromere and the third appendage are unpaired, but the tips of the second appendage are just visible as two minute papillae, at the summit of a great bilobed projection.

A comparison of this appendage with the third median pair in Fig. 91 shows that *each new median appendage divides first at the apex, the separation gradually extending toward the base. This, it will be observed, is the exact reverse of what occurs when the appendages of the right and left sides unite to form a single median one.* Compare Figs. 42, 43, 48, and 49.

In the median line, at the anterior end of the double embryo shown in Fig. 90, is a small depression in a dark mass of cells. The pit probably represents the common *Anlage* of the dorso-ventral muscles, which are seen to the right and left of each head, reaching the surface ectoderm near the apex of the optic ganglion.

In Fig. 91 the separation of the two heads has, by the wedge-like ingrowth of the two new halves, been carried down to the fifth thoracic metamere.

There is perhaps an actively growing point at the apex of the V, which gradually works backward, thrusting the old halves apart. But there is no indication whatever in surface views of such a proliferation, for each new part has the appearance of being as complete in every detail as the corresponding organs in the old halves.

My sections of double embryos were not perfect enough to be

of much assistance. I doubt, however, whether the most perfect sections would indicate any materially different condition from that seen in the surface views. It is somewhat surprising that the tension necessary to push the old halves over the yolk does not produce in them any other distortion than a gentle curvature to right and left. It shows how truly each part assumes its characteristic forms, dominated by its inherent structure rather than by the mechanical stress of adjacent organs. For example, in swinging the head of either embryo to the right or left, the movement may coincide on one side with certain lines of growth, but be directly opposed to them on the other. This may be seen in the diagram, Fig. 7, where it is obvious that the left side of the right-hand embryo is being swept along in the direction of its own lateral growth, and it must receive some additional impulse with which to overcome the resistance to it. But on the right side of the same embryo the movement is against the line of lateral growth. The internal stress at the lateral *C's* is very different from that at corresponding points in a single embryo, and very different from what it is at the median *C*, and yet the resulting organs under these diverse conditions are the same!

The reason these varying mechanical conditions have so little effect on the form of the organs is probably because they are so transitory. They differ essentially from the permanent and gradually increasing stresses that produce concrescence and degeneration.

In Fig. 92 the separation into two embryos began at an earlier period and is carried much farther than in the ones just considered. The head of each embryo has been swept over an arc of 90° . Further movement in that direction is prevented by the interference of the lower margins (as the figure stands) of the old mesodermic area. It is probable that the tension produced by this interference would about equal the devaricating force in the new halves, as soon as the two embryos formed a straight line tail to tail. If this condition is reached at an early period, the same forces, *i.e.* the tendency of the posterior margin of the mesodermic area to concresce behind each embryo, will ultimately force them apart. But

if the embryo is well developed before division takes place, the head ends will meet each other on the side of the egg opposite to the tails and will thus tend to prevent their further separation.

In Fig. 93 separation of the two embryos has taken place, as shown by the arrows, in a manner similar to that in Fig. 92. But before the new sixth thoracic appendage and those of the abdomen were produced, median fusion and antero-posterior degeneration took place in the left-hand embryo in the manner so frequently observed in single embryos. See Pl. V. The cephalic lobes have disappeared, and the first three thoracic metameres have fused in the median line, leaving nothing but a minute pit to represent the second pair of appendages, and a small, median papilla to represent the third and fourth.

In Fig. 94 the same process is carried further. In the way the egg now stands, the common axis of the two embryos was originally nearly vertical, as in Fig. 95. The lower one then moved upwards past the left side of the other one to its present position. It now occupies the free surface of the yolk between the dorsal margins of the right-hand embryo. Median fusion and antero-posterior degeneration then followed, whether before or after separation cannot be determined, reducing the left-hand embryo to the posterior part of the thorax and the abdomen. The latter has a very conspicuous tail lobe similar to that in Pl. V, Fig. 48.

It is seen on comparing Figs. 90, 92, and 98, that one of the hearts and tail lobes must have belonged to the original embryo; the others must be entirely new. For example, in Fig. 92 the heart and tail lobe just above *y* will be formed by the concrescence of the right and left margins of the old embryo, while the heart appearing at *x* will be entirely new. The two sides are so much alike in Fig. 97 that we cannot tell which is the old half and which the new. Now if the tail ends of these embryos should grow past each other, as in Figs. 94 and 96, then one embryo would carry off, according as it passed to the right or left of the other, either a new tail and a new heart, or the old ones. If we knew whether the heart of the original embryo in Fig. 97 was to the right or the left, as the figure now

stands, we could tell which was the new tail and which the old. This can be done in Fig. 96, for it is obvious that both halves of the tail lobe of embryo *A*, probably as far up as the last left thoracic appendage, are those of the original embryo. Embryo *B*, however, has carried off an entirely new tail lobe. The result is that the right half of embryo *A* will consist of the right half of the original embryo, and all its left half will be a new formation, except the abdominal part. In embryo *B*, its left half is that of the old embryo, except the posterior end, which is new, and was probably produced by the backward regeneration of that half of the body. Its right side is entirely new. A similar condition must prevail in Fig. 94, except that embryo *B* has here carried off the *old* abdominal lobe, and *A* the new one.

These conditions are best seen in the diagrams, Figs. 8 and 9, where the shaded portions represent the old parts, and the light ones the new. In Fig. 9, embryo *B* pushes past the left side of *A* and carries off a new heart and tail lobe, and the posterior portion of the abdomen on the left side.

In the well-balanced condition seen in Fig. 97, it is unlikely that further changes in the relative positions of the two embryos would take place. But if they should be forced to grow past each other, a different proportional combination of old and new elements would be produced from that in Fig. 96, and the cause of this would be due, in part at least, to the different periods in the formation of the new halves at which the embryos separated from each other.

In Fig. 95 the two embryos, arising by longitudinal fission as in the preceding ones, are still united tail to tail, but degeneration of the lower one has progressed so far as to reduce it to a slipper-shaped thickening with a depression in its centre, from which arises a papilla, probably representing the last trace of the fused sixth pair of thoracic appendages. A dark-rimmed depression in front of this probably represents the remnants of another pair.

In Fig. 98, division has produced two nearly complete embryos, the new half of the abdomen of each embryo being absent. The lower embryo has already begun to degenerate,

and presents a very good case of transverse fission, accom-

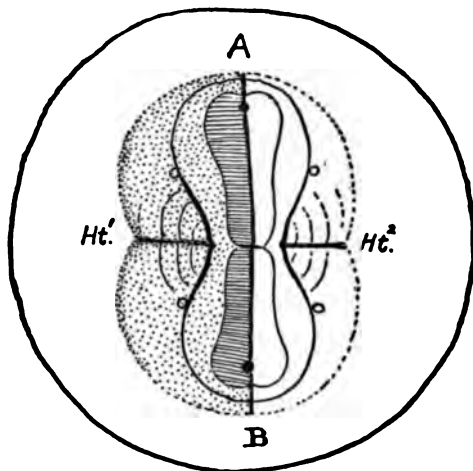


FIG. 8.

Diagram to illustrate the probable proportional composition of double embryos out of old and new parts.

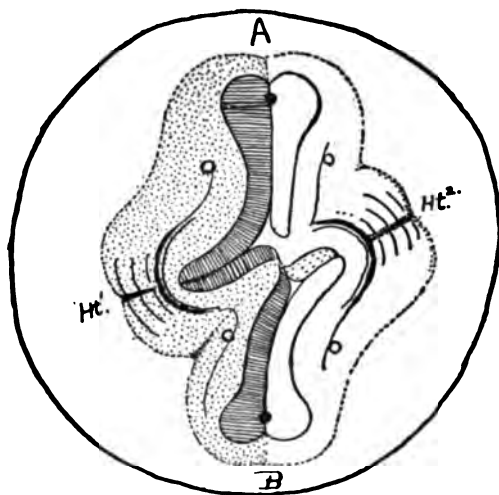


FIG. 9.

Diagram to illustrate the relation of old and new parts in the abdomen of separated double embryos

panied by median fusion. There is a marked "weakness" of the new half of the upper embryo, shown by the absence of

the second, and the small size of the invaginated fourth, appendage. There is no indication of segmentation lateral to these appendages, and the left half of the neuromere opposite the fourth appendage is apparently absent. The weakness of this half is further shown by a gentle curvature of the head toward the left, although at this stage in other double embryos it is curved to the right.

In Fig. 97 is a very late stage (about the trilobite stage) of a double embryo. The two embryos are tail to tail in a straight line, and so symmetrically developed that there is no indication whatever of the direction in which the two embryos separated from the primitive median plane. Median fusion and antero-posterior degeneration of each embryo has progressed as far as the fourth thoracic segment. As indicated by the large segmental sense-organ and the appendages, fusion and degeneration have progressed farther in the lower embryo than in the upper one. Interesting features of these two embryos are the two perfect hearts and tails, extending laterally at right angles to the continuous nerve-cords, and the large flabellae, which look like a separate set of appendages.

In Fig. 96 is what appears to be a case of division of a different nature from those just described ; but a careful examination will show, I think, that it is after all the same. We can easily and satisfactorily explain its condition by assuming that longitudinal division gave rise to two embryos in a straight line tail to tail, and that they separated and pushed past each other in opposite directions. The anterior end of the right hand embryo is turned vertically downward and has undergone median fusion and degeneration. This resulted in the fusion of the fourth and fifth pairs of thoracic appendages, leaving the sixth pair and the abdomen in a nearly normal condition. The abdomen, as indicated by the dotted line, has finally been thrown sharply to the right by the growth of the left side of the thorax of the larger embryo. The result is an embryo much like that in Fig. 97, only its longitudinal axis is bent at right angles, the anterior portion of what is left being in its primitive position, parallel with the axis of the larger embryo.

In Fig. 100 is a much older embryo, with the remains of a second rudimentary one attached to its right side. Separation of the two embryos probably took place in the direction indicated by the arrows,—the left-hand embryo undergoing median fusion and antero-posterior degeneration. The remaining appendages are so twisted and obscure that their identity could not be certainly determined. They appear to represent the fused third, fourth, and fifth pairs of thoracic appendages arranged in single line. The sixth pair are fused at the base, leaving the ends free. The first two pairs of abdominal appendages have also fused, something that rarely occurs with them, to form two median, tongue-like projections, each of which is bent almost at right angles.

On examination from the dorsal side, Fig. 101, the outlines of the two embryos are distinctly seen. The median dark streak in the smaller embryo probably represents the remnants of the oesophagus, or perhaps the heart. The large semi-lunar band of cells consists of the *fibre cells* that I have described elsewhere, and represents the coneresced margins of the mesodermic area. It should lie on the anterior dorsal surface of the thorax, but is here thrown forward towards the ventral surface.

The only instance observed in which there seems to be a deviation from the method of forming double embryos, just described, is shown in Fig. 99. This embryo was accidentally destroyed before a finished drawing of it was made, but it had been completely outlined and carefully studied. There is no question therefore about the correctness of the details of structure, as far as they are given.

At first sight the right-hand embryo appears to have arisen by longitudinal regeneration from the left side of the larger one. Aside from the fact that no indication of such a process has been seen elsewhere, it is difficult to imagine in detail the method by which it was brought about. It is not necessary to discuss these possibilities, as long as we can reduce this type to the usual one by assuming that the form of degeneration frequently seen in single embryos, has affected one of the embryos almost coincident with its separation from the other.

For example, if fission progressed in Fig. 91 down to the abdominal region, leaving the newly formed abdominal appendages as unpaired organs, just as the fourth appendage now is ; and if the anterior part of the right-hand embryo underwent median fusion and antero-posterior degeneration up to the fifth thoracic metamere, we would then have a condition like that in Fig. 99. I see little reason to doubt that the embryo in question was formed approximately in that way.

2. TRIPLE EMBRYOS.

Illustrations of triple embryos are seen in Figs. 102 to 104. The steps by which they were produced were probably as follows : It is assumed that in the beginning there was a single, normal embryo, and that it gave rise, by longitudinal fission, in the manner already described, to two embryos, each one composed of a new half and an old one, Fig. 7. The right-hand embryo then divides in the same way as the first, by the formation of two new halves, Fig. 10. The result is that the halves of the original embryo are now separated from each other by an angle of about 240° . The second embryo, *B*, is an entirely new formation, but embryo *A* consists of the original right half plus a new left half, and embryo *C* consists of the old left half plus a new right half.

The original line of concrescence of the posterior margins of the mesodermic areas, *Ht.*¹ (along which the heart is formed), remains unchanged, except in its position on the yolk, just as the original line of concrescence becomes the lower one in the double embryo. The other two heart lines, *Ht.*² and *Ht.*³, are *entirely new formations*.

If we may speak of the new halves as separate generations, their relations to each other in a triple embryo are as follows : In embryo *A* the body consists on the right side of the right half of a mother, and on the other of the left half of a daughter. Embryo *B* is composed on the right of the right half of a daughter, and on the left of the left half of a granddaughter. Embryo *C* is composed on the left side of the left half of a mother, and on the right side of the

right half of a granddaughter. The line of concrescence of the heart lines will have at $Ht.^2$ on one side the right half of one daughter, and on the other the left half of another daughter. These two halves cannot be said to belong to the same daughter without assuming some preformation jugglery by which the molecules of one half were shifted to the wrong side of the other. At $Ht.^3$ there is on one side of the concrescence line the margin of the right mesodermic

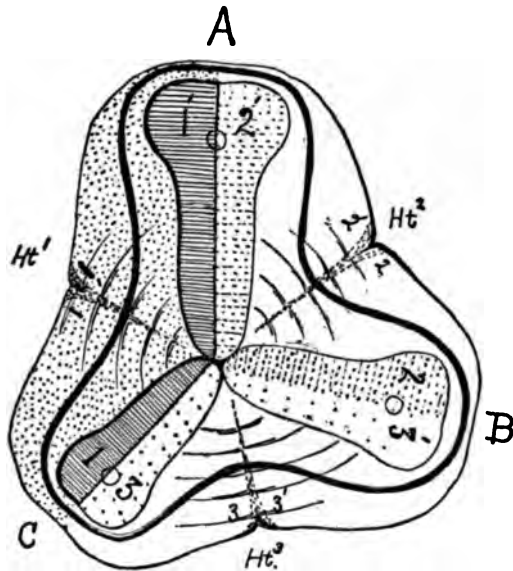


FIG. 10.

Diagram of a triple embryo, to show the relation of the old and new parts. Original halves, 1 + 1'; second generation, 2 + 2'; third generation, 3 + 3'.

area of a granddaughter, and on the other the margin of another granddaughter. At $Ht.^1$ the concrescence line has, on either side, in their original relations, the right and left margins of the mesodermic area of the mother. We may therefore call the hearts, or other organs developed along these three lines, respectively the mother, daughter, and granddaughter hearts, etc.

In explaining the condition of the triple embryos in Figs. 102 to 104, we shall assume that the original embryo divided

lengthwise in the manner already described, producing *A* and *BC*, and that *BC* divided, giving rise to *B* and *C*. In Fig. 102 *A* remains practically normal; *B* has undergone median fusion and transverse fission across the line of the fourth segment. The abdomen and last two thoracic appendages are practically normal, while the anterior part of the thorax and cephalic lobes have disappeared, except one pair of fused appendages.

Passing around to embryo *C*, we find median fusion and degeneration have obliterated everything but the abdomen and the last pair of fused thoracic appendages.

In Fig. 103, *A* has undergone median fusion and degeneration, forming a pretty good example of an hour-glass embryo. The same process has affected *B*, obliterating entirely the cephalic lobes and anterior portion of the thorax. The dorsal organs, however, are not quite fused in the median line. But this has taken place in *C*, and in other respects the degeneration is carried farther than in *B*. In both these triple embryos, then, *the path of increasing degeneration is that of a spiral from A to C.*¹

In Fig. 104 all three embryos are reduced so nearly to the same level that it is hard to determine which is the most degenerate. They are reduced to the last two fused thoracic appendages and a remnant of the abdomen. Embryo *C* has the smallest appendages and may be taken to be the most degenerate. But between *A* and *B* there is so little distinction that one cannot determine whether the line of degeneration follows a right or a left handed spiral. We shall return to this point later.

Discussion of Observations on Defective and Exuberant Embryos. — There is little hope in the present condition of our knowledge of finding anything like a satisfactory explanation of the phenomena of either defective or exuberant embryos, because the solution of the problem is bound up in that of vitality. While the futility of seeking final explanations of vital phenomena is fully recognized, we have ventured, supported by the facts on variation here described, to approach some of the outposts of the subject. These facts are numerous and in some

¹ Possibly, from *C* to *A*, as more recent evidence indicates.

instances novel, and it will probably be admitted that they are somewhat conducive to speculation. I shall make them alone the basis of the argument which follows. There is an obvious advantage in treating the subject in this way, for the chances of misconception due to confounding unlike results or conditions with one another are thereby reduced to a minimum.

We may assume that the three embryos of triple monsters are endowed at the outset with equal potentialities and that it is merely a question of time that will bring them all to the same condition of degeneration. But one embryo must be older than the other two, both of which are of the same age. If the age of the embryo, that is the time that has elapsed since it became an independent embryo, determines the amount of degeneration, then *C*, which is the most degenerate, ought to be the oldest ; and *B* and *C*, being of the same age, ought to show the same degree of degeneration. But as this is not the case we must assume that some other factor than the time each has had to degenerate determines the degree of degeneration. We may also dismiss as possible factors the environment, for as we have seen that was the same for all classes, yet, in spite of that, defective, exuberant, multiple, and normal embryos were produced ; and also that when the conditions of development were made excessively abnormal, no abnormal embryos were to be found.¹

In discussing the phenomena of multiple embryos as well as the other variations described, we must bear in mind the following facts, which although apparently contradictory in some cases, must nevertheless be made to harmonize and be mutually confirmatory before any approach to an explanation is possible. These facts are :

(1) Whatever variations are here considered are probably due primarily to structural variations resident in the ovum, and not to differences in the environment.

¹ It is obvious that we should find at least as many abnormalities under the abnormal condition as under the normal, especially if the primary cause of the variation is to be sought in the eggs themselves. The absence of abnormalities under the former condition is probably due to the fact that under the prolonged drastic treatment to which they were subjected, only the normal healthy ones survived.

(2) There is a great difference in the growth period under apparently the same conditions.

(3) There is a great difference in the size of different embryos, some being much larger than the normal and others smaller.

(4) Certain organs or regions of the body may be entirely absent and are not subsequently restored.

(5) When organs once formed disappear in certain regions, it is usually by median fusion and degeneration in the reverse order of their age and specialization.

(6) Multiple embryos are due to the formation of new parts, which appear in the reverse order of that in which old organs disappear by median fusion and antero-posterior degeneration.

(7) Multiple embryos thus formed quickly disappear again by median fusion and antero-posterior degeneration.

(8) Individuals of triple embryos recently formed differ greatly in size and in the amount of degeneration.

(9) In old triple embryos the individuals are more nearly alike.

Taking up each set of facts, except the first, it would seem from a consideration of the variations of the second class that every individual and every part of it has a definite *rate* and *range* of growth, a rise and a decline like a time clock that has been set to go a definite period at a definite rate. The time for the whole embryo may be reduced apparently to almost any fraction of the normal one, as in those that die a natural death before reaching stage *C*; or individual organs may die and disappear by median fusion and degeneration before the other organs have completed their development; and defective parts either remain defective, or dwindle and disappear in the midst of plenty, side by side with healthy flourishing organs. Embryos six or eight months old are found in the same stage, to all appearances, as normal ones only six or eight weeks old. Both these kinds of variation are apparently best explained by assuming a primary variation in the quantity or quality of growth material, or of both. Either variation may express itself as a variation in the intensity of the "growth force" that may be measured in terms of the range of development, that is, the number of intermediate stages produced, or

in terms of the rate. The original variation may be local or general, but in either case, the deficiency, if there be one, is not restored by nutrition, or by drafts on a general supply.

(3 and 4). Variation in the size of separate organs or of the whole embryo, or the entire absence of organs, seems to be due to a variation in the amount of formative material of that particular kind out of which the variable organs are formed.

The variation in the amount of formative material expresses itself in these cases in a variation in the size of the parts, not in their rate or range of development. The formative material when diminished or absent does not appear to be restored or replenished, because regeneration of defective or absent parts does not take place, although the organs that are present find plenty of material with which to continue their own growth.

The thing then that is lacking in this case seems to have a definite location and is not distributed throughout the embryo. The deficiency is due apparently to the absence of some kind of formative material, and not merely to a diminution of its formative powers or to a variation in the quality.

(5). Organs once formed frequently disappear by median fusion and degeneration, *in the reverse order of their age and specialization*. In other words, on a given segment the right and left organs telescope into each other at the median line, and disappear one after the other in the order of their position and original formation. This mode of degeneration must be due to some inherent structural conditions, and not alone to mechanical stress or tension, because it always occurs in the same way at various but well-determined places, where the mechanical stresses due to growth of the surrounding parts must be quite different. We may assume that the reason the median end of a half segment disappears first is *because it is most specialized or most highly developed, and therefore most likely to feel the effects of diminished vitality and increased tension*. The other parts follow in order for the same reason, but the reason the latter move bodily toward the median line is because a path of least resistance is constantly reestablished there by the degeneration of the organs nearest that point.

(6). Multiple embryos are produced by the formation of new parts which appear in a definite sequence and in a definite place, and in a manner the reverse of that by which old organs disappear by median fusion and degeneration.

This result may be attributed either to the presence of an excess of formative material, or to forced drafts on the reserve brought about by some unknown internal conditions: If the former supposition were correct, there is no obvious reason why the additional parts should not be permanent acquisitions. We cannot assume that such an arrangement of parts as we see in multiple embryos is necessarily fatal to their coördination, because the large numbers of double embryos of all kinds that have reached advanced stages of development, as is well known, would testify to the contrary. On the other hand, our next fact (7), that multiple embryos almost immediately degenerate by median fusion and antero-posterior degeneration, shows, it seems to me, that *there is no production of new "formative material," but a misdirection of that already existing. There is in some way the dividing up of the sum total of formative material so that it crystallizes out along two or three lines instead of one.*

The fact that these new centres start out all right but soon begin to degenerate in the same way that poorly endowed single embryos do, shows that there was not enough formative material to go round; that multiplying the formative centres simply cuts off specialization and longevity at the other end.

This supposition is still further supported by the next two facts, namely (8) :

The individuals of triple embryos recently formed differ greatly in size and in the amount of degeneration, and (9) In old triple embryos the individuals are more nearly alike. Before we consider these two points further, let us assume for a moment that in the formation of multiple embryos there has been, figuratively speaking, a forced growth producing material faster than it can be differentiated, and that 'the point of greatest differentiation tension is at the oldest point, where the most growth and specialization had already taken place.

This point, as we have already shown, is the median part of the most anterior end of the body. The tension there would be most likely to be relieved by the production of a new organ at that point, exactly duplicating in size and differentiation the one side of which it was formed. If the differentiation tension, if we may use the term, is still too great, it will be relieved by the production of more new organs at the next point of greatest differentiation, namely, at a point lateral to the two newly formed organs, and on the median side of the corresponding organ in the next posterior segment. The process might go on till two entirely new halves were produced in that way, or it might stop at any time that the extra tension was relieved.

This supposition may in a measure account for the remarkable fact that the newly formed organs do not pass through the various stages of development the other organs did, but assume at once whatever characters the old organs may have at that time. For example, the tip of the new unpaired appendage in Figs. 90 and 91 is just as perfect in form and character for that age as the other appendages on the old halves, and for every successive part of it that appears, it is the same. It looks as though the perfect appendage had been previously concealed in the yolk, and gradually rose to the surface point first, till completely exposed.

If two entirely separate embryos are produced by this forcing process, the growth force, if it is an exhaustible quantity, will be irretrievably subdivided between two embryos. Each will have half the energy of the parent, provided there has been an equal distribution between the new and old halves. But on the formation of a triple embryo, the undivided one will have twice the energy of either of the other two.

On the other hand, if there is at first no recuperation of embryo *B* from *A*, or *C* from *B*, the latter must contain the least amount, and *C* less than *A*, *provided* there is recuperation of the weaker half of each embryo from the stronger during the process of division. This is clearly in accordance with their degrees of degeneration, as shown in Fig. 102. But if we suppose that there is subsequently a slow diffusion of formative

energy throughout the three embryos, so that each one gets an equal share, there will be a tendency to bring all three embryos down to the same grade of degeneration. We may thus explain the diminished difference between the three embryos in Fig. 103 and those in 102; and finally in Fig. 104, where the three embryos have evidently been formed a long time, all three are reduced to nearly the same condition. At first sight these conclusions seem to be in direct opposition to the fact that in single asymmetrical embryos *there is no indication whatever that the stronger half possesses any power by virtue of which missing parts on the opposite side of the median line can be restored.* If one embryo can produce two new halves by drawing on its bank account, as in the formation of double and triple embryos, why do not defective single embryos restore an absent half or quarter? We can only say that in such cases the right half of the embryo, for example, was absent because the formative material for that part was absent from the start. Theoretically there is no reason why the lost part should not be restored by a forced draft on the corresponding organs of the whole side. *But there is no particular reason for assuming that forced growth would be likely to occur in an embryo that was already defective in growth material.*

On the other hand, it is a point to be borne in mind that in all the defective embryos shown in Pl. IV very few show a defect at the anterior end of the body, unless, as in Fig. 38, the whole left half is absent, or in Fig. 37, which is probably a double embryo. The parts most frequently absent are the posterior ones, or ones across the middle of the thorax. *That is, they are the parts least likely to be restored from the opposite side, provided their restoration took place in the same order that the organs of new halves are formed in double and triple embryos.*

According to Hertwig and others, the stimulus of a modified environment alone is sufficient to call forth the new embryo. But as in the cases we are considering the environment was nearly the same for all, it can only mean that out of many thousands of eggs *the few that produced double or triple monsters were different at*

the outset, and for that reason responded in a different way to the same environment. Again, if it is the environment alone that produced the excessive growth manifested in the formation of two or three embryos out of one, we should expect that under these favorable conditions the new embryos would be large and vigorous, but as a matter of fact they are not, for no sooner are they once formed than they begin to degenerate, and finally all of them may be reduced to mere remnants.

Hertwig's criticism of Weismann's explanation of polymorphism in ants and bees, as well as of multiple embryos, is, it seems to me, a valid one. For, as he points out, we have no right to assume that the embryo is provided with several sets of germ plasm destined to develop, under proper stimuli, into new embryos or organs, unless the necessary stimuli are likely to occur in nature. And it might also be added that this condition could not have been fixed by natural selection, if its realization brought death with it.

On the other hand, the assumption that Hertwig makes, that the stimulus of a new environment is alone sufficient to produce new formative material, is not a necessary one. Indeed, the facts seem to me to point to an opposite conclusion.

The factors producing forced growth of this kind, it seems fair to assume, are the reverse of those that cause median concrescence, because the sequence of events is nearly the reverse. That is, excessive tension causes organs to fuse and disappear along the median line. Is it not probable then that abnormal reduction of this tension would facilitate the formation of new organs there, which will then reverse the tension conditions and cause the organs to disappear?

The formation of multiple embryos may be the result of some inherent defect in the ovum, and the stimulus of a modified environment may exaggerate these defects, and increase the percentage of multiple embryos; but there is no evidence to show among the higher, segmented animals, at least, that double or triple embryos may be produced by the sole action of any definite environment.

The fact that double and triple monsters appear under abnormal conditions, is not a valid argument against the mosaic

theory, or, at least, not in its widest sense, but only against the rigid limitations placed on it by Weismann and others.

It is well known that an embryo may, under stress of new environment, divide and produce two or more new ones ; and perfectly formed embryos are produced from fragments of segmenting eggs, but these embryos have *suffered either a diminution in size, or in vitality, or both*. No one, so far as I know, has succeeded in raising a fraction of the original ovum into a mature individual showing the same longevity and vitality as those raised from whole ova. The same is true of double or triple embryos produced by excessive growth.

If we assume with Weismann that the material for two or more embryos is present in each egg that is capable of producing multiple embryos, and that the stimulus of the environment has called them all into activity, it will be difficult to explain how it happens that the newly formed parts disappear so soon after formation, and also—a difficulty that, so far as I know, has not been foreseen before—*why the new material should be in isolated halves of different individuals instead of in one or more entirely new individuals distinct from the old*, or why these halves should develop and unite with the old ones in the way they do.

It is probable that the same method of forming the new parts in multiple embryos of *Limulus* is followed in other animals, only it has not been recognized there, because the embryological processes are more obscure.

It should also be observed in this connection that there is no evidence that the new organs come from some indifferent reserved cells. In addition to the fact that, under very favorable circumstances, no trace of such cells can be seen, it would be difficult to explain why they should always first manifest themselves at the point of greatest specialization, that is, at the very anterior median line, and grow backwards instead of forwards. We should, on the contrary, naturally look for them at the posterior end, and expect that they would produce a new embryo there in the usual manner.

It seems to me that we must assume that in all normal ova there is a definite quality and quantity of formative material

that will under normal conditions produce at the end of a certain time an animal of a given form, capable of performing a number of activities. The capital each ovum starts with determines the result, if the conditions are normal. A change in environment may retard or accelerate the mechanism ; it may throw certain parts into new tracks ; it may influence the distribution of formative material as a whole, but it cannot, within the period of one generation, change the nature of the formative material.

However the machine may vary, we recognize it as the same machine, — incomplete perhaps, but if so, we recognize the vacant places. In very rare cases (only one observed) a *Limulus* embryo may have more than twelve thoracic appendages, but the extra appendage is exactly like one already existing. In no case is there found a new organ or part different in kind from those already existing, and in no case is an organ out of place in reference to others. The chelicerae always come back of the cephalic lobes ; and a flabellum, if it is present at all, always occurs on the outer margin of the sixth pair of appendages. We can only attribute the original absence of an organ, or of any part of the embryo, and the subsequent failure to reproduce that organ, to the original absence or diversion into other channels of the material out of which that organ was to have been formed.

We can explain the formation of double and triple embryos, not on the assumption that the original formative material has been increased, but that it has been divided and diverted into separate channels, and the consequent diminution in the quantity available for the new embryos has been one of the causes of their subsequent degeneration.

There are in normal embryos inherent lines of weakness along which there is certain to be diminished growth. They mark off the different regions of the body from each other ; and when through the action of the environment or through congenital conditions there is a diminution of vitality, it is shown by the diminished size of these regions and by a tendency to fuse along the median line.

Increased vitality is shown by increased size, persistency of form, and by the production of new organs in the reverse way of that in which they disappear. If the increase is due to the original presence of an increased amount of formative material in the ovum, the increase in size, vitality, and number of organs will be a permanent characteristic of the individual ; but if it is due to the accelerating action of the environment, there will be a corresponding loss at some other time or place. We may further conclude that :

(1) In multiple embryos of *Limulus* the new organs are formed by forced drafts on the old material.

(2) That the new halves are weaker in formative power than the old.

(3) That equality is established by the interchange of material from the stronger to the weaker halves.

(4) That at first the sum total of formative energy in both halves of embryo *C* is less than in *B*, and in *B* than in *A*.

(5) That equality is finally established between all these embryos by interchange of material, so that in the end all three are reduced to the same grade of degeneration.

(6) In defective single embryos, the absent parts are absent because their specific formative material was absent. Theoretically the absent organs might be restored by forced growth of the other side. But there is no reason to expect forced growth will occur in an embryo already defective in formative material. If it does occur, it will more likely be at the anterior end, and of course the restoration will not be detected. If it does occur in that way, it explains why unilateral defects are more frequently seen at the posterior than at the anterior end.

VII. DEGENERATION AND DEATH OF *LIMULUS* EMBRYOS.

The causes of degeneration and death of the embryos described in the preceding sections are due to abnormal limitations in either the power of division, specialization, or longevity of the cells, or to various combinations of the same.

The structure of a fully developed adult animal, it seems to me, must depend on the relation that exists between (1) the rate of production of new cells, (2) the rate and the amount of specialization of these cells, (3) the longevity of the completely specialized cells, and (4) the death rate of the cells, or their rate of decline toward a simpler, less specialized condition. The interrelations of these factors must be extremely complex and to a certain extent independent of each other; for reproduction, specialization, and decay may, apparently, take place simultaneously in any part of any organ, or in the entire organism, in almost any conceivable proportion.

An essential feature of organic death in the higher animals is the cessation of normal activity in many different organs, because other organs, by accident, or by inherent conditions, cease to perform some particular work, perhaps insignificant in itself, upon which all the others depend.

The nerve cells, for example, may be performing faithfully and well their particular work, and yet may perish for lack of proper nutrition, or owing to the presence of poisonous substances that should have been eliminated from the body. We do not know how long they might have continued to act under favorable conditions.

Every living thing has a more or less definite size, form, life period, and kind of activity. It is apparently assumed by some writers that these manifestations are not merely pre-determined by the organization of the ovum, acting under the guidance of a changing environment, but that there is an actual deposit of formed material corresponding in some way to every one of the almost infinitely numerous parts of the future organism. That there are definite potentialities in every ovum cannot be denied, but these potentialities must not be confounded with what actually exists as specific matter, and which forms the actual physical basis of the ovum at a given time.

The future organism is the resultant of the action on an initial, specifically constructed mass of forces outside of and independent of the mass (1) of such forces acting on the newly formed material added to the old, and (2) on the continued interaction of these masses and forces on one another, under

constantly changing conditions brought about by these interactions. To affirm that the resultants of such complex interactions can be in any sense preformed is grossly inaccurate and misleading.

To use the term "preformation" to designate in any way the remote antecedents of a bit of living protoplasm is like asserting that the germs of a banner cloud are preformed in the south wind, or that the germs of a watch are preformed in the iron and coal used in making it.

Embryological processes are not to be interpreted merely as necessary preliminaries to a higher condition. There is no beginning or end. Each phase and part of a living thing should be treated as it actually is, and be accorded its full value as a perfected, completed thing, — not as it is going to be, ignoring the present to catch some imaginary glimpses of the future.

In the higher animals, death comes to the organism in most cases, it would seem, as a result of the increasingly complex interrelation of cells. Non-living compounds accumulate in the tissues with age and destroy their elasticity or permeability; the calibre of conducting tubes is diminished and they fail to furnish the necessary supplies, or excessive demands lead to rupture, lack of coördination between volume and surface exposure, excess of capillary friction accompanying increased size of organs, etc. Such slight defects may place insuperable barriers to further growth and specialization. The same may be true of the individual cells themselves. We need not assume that they cease their activities at the end of a certain period because a certain amount of inherited energy has been liberated, as in the uncoiling of a watch-spring, but because they cannot clean themselves or adjust the necessary repairs to the new conditions. Environment, within and without, winds up and liberates the springs, not the ancestors. As long as the environment does this, the vital mechanism will continue to go till stopped by the products of its own activity, and the mechanical impossibility of adjusting old parts to new requirements.

The more complex the relation of parts and the more perfect their mutual adaptation, the more sure and complete will

be the collapse of the whole, when the working of one part is interfered with. Each organism by itself is a world of individual cells where heredity, use, disuse, and struggle for existence are the determining factors of form and function, just as in the larger world organisms as a whole are the resultants of these factors. In both cases, sudden and great changes in the environment result in a rapid collapse or death, owing to the impossibility of the cells on the one hand, or the organism on the other, adapting themselves to the new conditions; and the result is death of the individual organism in one case, or extermination of the species in the other. If the change is a slow one and one calling for less specialization, in fewer directions, degeneration, or reversion to a simpler plan of structure, may follow till perhaps something resembling the original starting-point has been reached.

It is an exactly analagous process to this that occurs in some of the degenerating embryos of Limulus. It is a new kind of death for an individual organism, or, at least, not like the one with which we are familiar. The embryo, a community of thousands of different kinds of cells, does not die like a nation swept by a pestilence, or like the starving inmates of a helpless vessel, but like a flourishing community in the midst of plenty, where some of the infinite niceties of adjustment are such as insensibly to reduce the birth rate below the death rate, to reduce the complex interrelation of individuals, until after many generations the last survivor, reduced to the lowest terms, disappears.

The point we wish especially to emphasize is that in the degenerating embryos of *Limulus*, cell reproduction, cell specialization, and cell decay are progressing side by side in every part of the body. Karyokinetic figures and the fragments of decaying nuclei are found side by side. Whether the animal develops or degenerates depends on the relative intensity of these three factors. The embryos dwindle in size because the death rate of the cells is greater than the birth rate. The embryo loses nerve centres, sense-organs, and appendages because the amount of specialization of individual

cells is cut down more and more, till only the simplest kinds remain, or because the new ones die before they become specialized. The lack of specialization may affect different parts unequally, as when the surface details of structure fail to appear on the otherwise well-developed appendages, as in Fig. 21, or the nerve-cords, the appendages, or sense-organs are lacking.

As we might expect, the process is never exactly the same, but it invariably tends to carry the organism back, in the main, over the old lines of progressive development, till it reaches its primitive condition, namely, a small community of untrained, insubordinate individuals, which in turn die one by one, till the death of the last survivor exterminates the race.

This may be called the true natural death of an organism,—all others are more or less catastrophic, due to the increasing lack of coördination and adjustment to the new conditions produced by its own growth and specialization. A natural death like this is only possible where the degree of specialization has been comparatively small, and where every cell may receive the stimulus and material necessary to the continuance of its activities and the discharge of its waste or noxious products.

If this be true, then there is no such distinction to be made as “mortal and immortal” protoplasm. All protoplasm is “immortal,” in the same sense that chemical compounds, or mixtures of the same will continue to be formed, manifest their specific properties, and disappear so long as the proper environment is maintained.

Cessation of vital activity, then, is due solely to inadequate environment, whether we are dealing with highly organized individuals, or with bacteria, amoebae, or human ganglion cells, or with any part of these.

The growth of the smallest protoplasmic part of a cell differs from the growth of a crystal in the fact that, having grown, the conditions of growth and persistency are more materially altered in the former than in the later case.

The metabolic changes that take place in and around the cell are to a certain extent processes of filtration. The vital pro-

cesses cease, not because some hidden spring within needs to be renewed, or because, like a spent rocket, it needs to be recharged, but because the still perfect mechanism is choked with dust and weighed down with uneliminatable material. Before the final collapse some virgin fragment of the original material escapes to begin the same process anew.

We need not assume that this new material, or germ, is essentially different from the living parts of the old machine. We certainly need not look on it as a wonderful piece of pyrotechnics, with countless preëxisting fuses, percussion caps, and hidden chambers, adjusted to discharge themselves at the right instant and in the proper sequence — a something which needs but the spark and afflatus of proper environment to start on its heedless career with infinite splutterings and explosions, to cease only when it becomes an empty shell!

There is in our opinion nothing to be gained by such a view of developmental processes. Nevertheless, in discussing such problems the use of figurative language cannot be avoided, for nothing more than a vague conception can be formed of the infinitely complex mechanism at work within the embryos even of the very lowly organized animals. To follow its normal course of action or development to the end, there must be in the ovum infinite niceties in the qualitative and quantitative adjustment of the new parts to the old; the chemical time-locks, that are to fix or release, must be set with exactness to the time and place, and the whole mechanism made adjustable to a rather wide variation in its surroundings. This being the case, who will venture to say that the entire absence of the third thoracic appendage was due to the fact that a certain particle, destined to grow into that particular appendage, was accidentally omitted from the chromatin of the segmentation nucleus?

Is it necessary to suppose because the third left thoracic appendage in *Limulus* was invaginated and its mate projected freely from the surface, that the former was derived from a peculiarly constructed molecule, or biophor, or whatever you please to call it, that was bound to develop into an invaginated appendage and not one projecting in the normal way? As well assume that a river inherits two kinds of sand grains, one of

such a peculiar structure that they give rise to sink holes, and the other to sand banks !

The facts of variation are of value to the morphologist as well as to the biological metaphysician. If, for example, invaginated appendages are formed, and are formed frequently, it shows that in that direction is, in its broadest sense, a path of low resistance likely to be followed again and again. The same is true of the deviations affecting the margin of the mesodermic area, and in fact any of the deviations that occur often and in a definite way.

In the inevitable shifting of internal relations that occur in all living organisms, this path is as likely to become a permanent path of least resistance as the other is to remain as it is. In other words, the normal and abnormal exchange places.

In conclusion, there seems to me no evidence in the variations here described to support the theories which attempt to explain heredity by assumptions that leave no room for the idea that the ovum is an *organism* — theories which make the ovum a mere receptacle to hold job lots of ancestral organs, to be shuffled together and dealt out again during segmentation by some archoplasmatic prestidigitator! It makes little difference whether the germules, plastidules, biophores, or whatever we may choose to call these corpuscular "brownies," come from immediate producers of the ovum, or from ancestors ten thousand generations back ; they are things, it seems to me, which exist only as mere names that help to bring before the mind a few of the factors in an extremely complex process.

EXPLANATION OF PLATE II.

Nearly all the figures were outlined with a camera and drawn to the same scale. They were made from mounted and cleared specimens, viewed with a raised condenser and wide open diaphragm. In this way they appear bright red on a clear yellow field; the elevations showing dark, the depressions light.

The embryos *A* to *E* represent normal stages introduced here for comparison with the abnormal ones. Figs. 1 and 2 belong to an earlier series, where a different method of designating the stages has been adopted.

FIG. 1, $\times 50$. Surface view of a normal embryo, stained and cleared in oil of cedar. The cephalic lobes form a clearly outlined, semicircular plate of ectoderm, thickened on each side. There is no trace of an oesophagus, but very faint indications of the cheliceral segment may be seen on the posterior margin of the lobes. The next three thoracic metameres are fully formed. In reflected light they appear, in surface views of opaque preparations, as gentle undulations of the surface, forming a series of ridges and valleys. The lateral ends of the low ridges are bent backwards and unite with each other near the margin of the mesodermic area. When the eggs are cleared in oil, the mesodermic segments are seen beneath, and about coextensive with the surface ridges. The fifth segment is just separating off from the anal plate. Along the median line of the latter is a slit-like invagination to form the *telopore*, from which a sheet of inner layer cells extends laterally and forwards, while beneath the invagination a line of cells extends into the yolk.

FIG. 2, $\times 50$. Surface view of an embryo about 10 days old. The drawing was made from studies of the opaque embryo in alcohol, and from studies of the same embryo cleared in clove oil and stained in borax carmine. The sixth segment has appeared on the anterior margin of the anal plate. The lateral ends of the four preceding mesodermic somites have become confluent, and the mesodermic area thus formed is provided with a distinct thickening along its lateral margins, *m. a.*, forming what I have called the "thickened rim of the mesodermic area."

The cheliceral segment is now quite distinct, and a faint, dark band is seen in front of the cheliceral segment connecting the two sides of the cephalic lobes.

FIG. 3, $\times 55$. Surface view of embryo about twelve days old, stained in borax carmine and cleared in oil of cedar.

All six thoracic segments are now formed. The sixth, as was the case at a corresponding age with each of the preceding segments except the chelicerae, is very plainly marked by its dark color, and by the sharp furrows that lie on either side of it.

The second, third, and fourth pairs of appendages have appeared, and on either side of them are faint spots, that are perhaps the beginnings of the sense-organs, seen in this region more clearly at a later stage.

FIG. 4, $\times 50$. Surface view of a normal embryo 14 days old, stained in borax carmine and cleared in oil of cedar. The three pairs of appendages are more conspicuously developed, the oesophagus has appeared at the anterior margin of the cephalic lobes, and the brain and ventral nerve-cords are now easily distinguished by the characteristic mottlings, due to the presence of many minute pits, precisely similar to those described and figured by me in scorpions. The appearance is due to the presence in the nerve-cord and cephalic lobes of numerous independent, bud-like thickenings of the ectoderm, which in histological special-

zation and in general appearance resemble sense-organs. The suggestion that the appearance is due to a folding to increase the surface, or that they represent "neuroblasts" (Wheeler) is inadequate, and both suggestions are based on misapprehension of the facts. We shall treat of them more fully elsewhere.

The rim of the mesodermic area is considerably enlarged, and is growing towards the median line back of the anal plate.

The telopore in this particular individual has disappeared, but the median streak of inner layer cells, derived from it, is still visible.

FIG. 5, $\times 60$. Surface view of a normal embryo 18 (?) days old. All the thoracic appendages except the first pair are now elongated processes having the characteristic shape of thoracic appendages. The sixth pair are still transversely elongated, and resemble in form the early stages in the development of the abdominal appendages. The most striking feature of this stage is, however, the series of large, shallow depressions on the outer side of the marginal fold, *m.f.* It is only in exceptional cases that the depressions are visible. They are most readily seen by reflected light in shelled ova, shortly after treatment with the hardening reagent, picro-nitric acid. The second and third, and the fifth and sixth, quickly disappear, leaving no trace behind. The first is a little smaller than the rest and lies a little in front of the chelicerae, but appears to belong to that segment. It develops into the lateral eyes. The fourth, *d.o.*, lies exactly opposite the fourth thoracic appendage, and growing rapidly, gives rise to the conspicuous organ, undoubtedly of a sensory nature, found in nearly the same position as late as the time of casting the first larval shell. The lateral eyes gradually move backwards till they lie on the dorsal side of, and posterior to, the so-called dorsal organ, *d.o.* The rim of the mesodermic area is decidedly thickened, and consists entirely of the remarkable cells containing a coiled fibre. Along the whole extent of this thickening, the inner and outer layers of cells are continuous; elsewhere, except along the median line, they are sharply separated. Sections show clearly that the inner layer cells along the entire length of the thickening are receiving extensive additions from an inward proliferation of the overlying ectoderm. The posterior margin of the mesodermic area is drawn out into two backwardly directed lobes, which frequently show traces of segmentation comparable with that seen in the early stages of the older somites (Fig. 1).

FIG. 6, $\times 41$. Surface view of entire ovum about 21 days old. Picro-nitric acid, borax carmine, balsam. The stained embryo is shown as a transparent object.

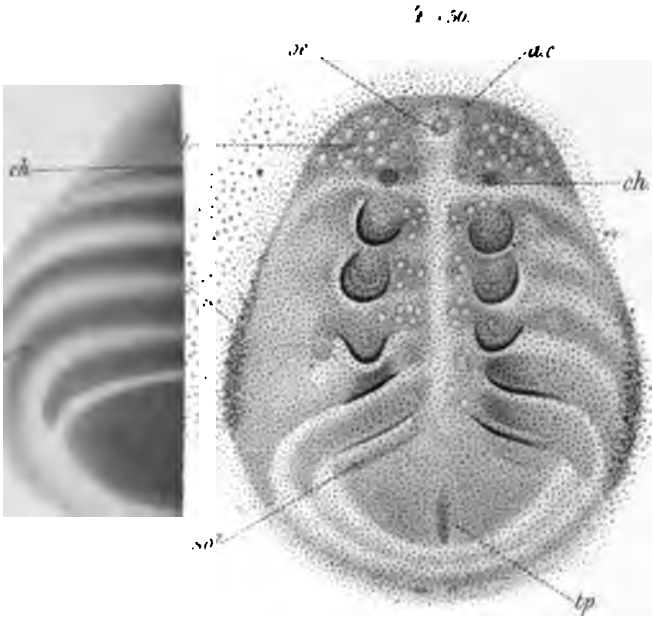
The operculum and first pair of gills are well developed, but the chelaria are as yet barely visible. The cephalic lobes have become specialized into the semi-circular lobes, *s.l.*, and the optic ganglia of the lateral eyes, *o.g.*; the remaining portions constitute the brain proper, *br.* The ectodermic thickening to form the corneagen of the median eyes is shown at *m.e.* The openings of the tubular invaginations of the median eyes and their nerves are shown at *p.e.t.* The fused distal ends of these tubes will form the median eye vesicle; the remaining distal third of the tubes is converted directly into the corresponding portion of the median eye nerve. The proximal portion of the nerves is formed by the separation of the nerve fibres from the peripheral surface of the tubes, leaving a collapsed epithelial tube behind, which persists for a long time after hatching as a functionless remnant. The separation of nerve fibres from the proximal ends of the eye tubes takes place before these parts of the tubes fuse with each other, so that the

distal end of the median eye nerve is unpaired, while the proximal end is paired. The roots of the Λ -shaped nerve terminate in the semicircular lobes.

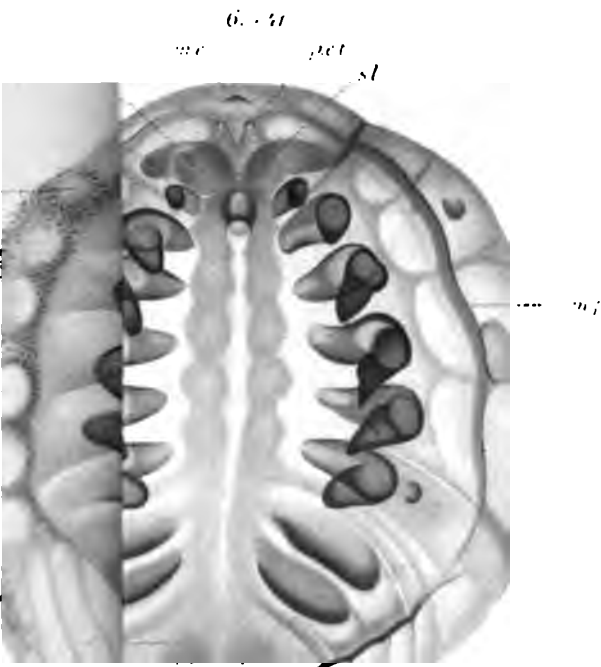
The lateral eyes have moved backward, away from their original position to the dorsal surface opposite the second and third thoracic appendages, *i.e.* The "dorsal organ" has retained its original position on the fourth segment. The dorso-ventral muscles, dividing the yolk into segments, and passing between the primary liver lobes, have appeared.

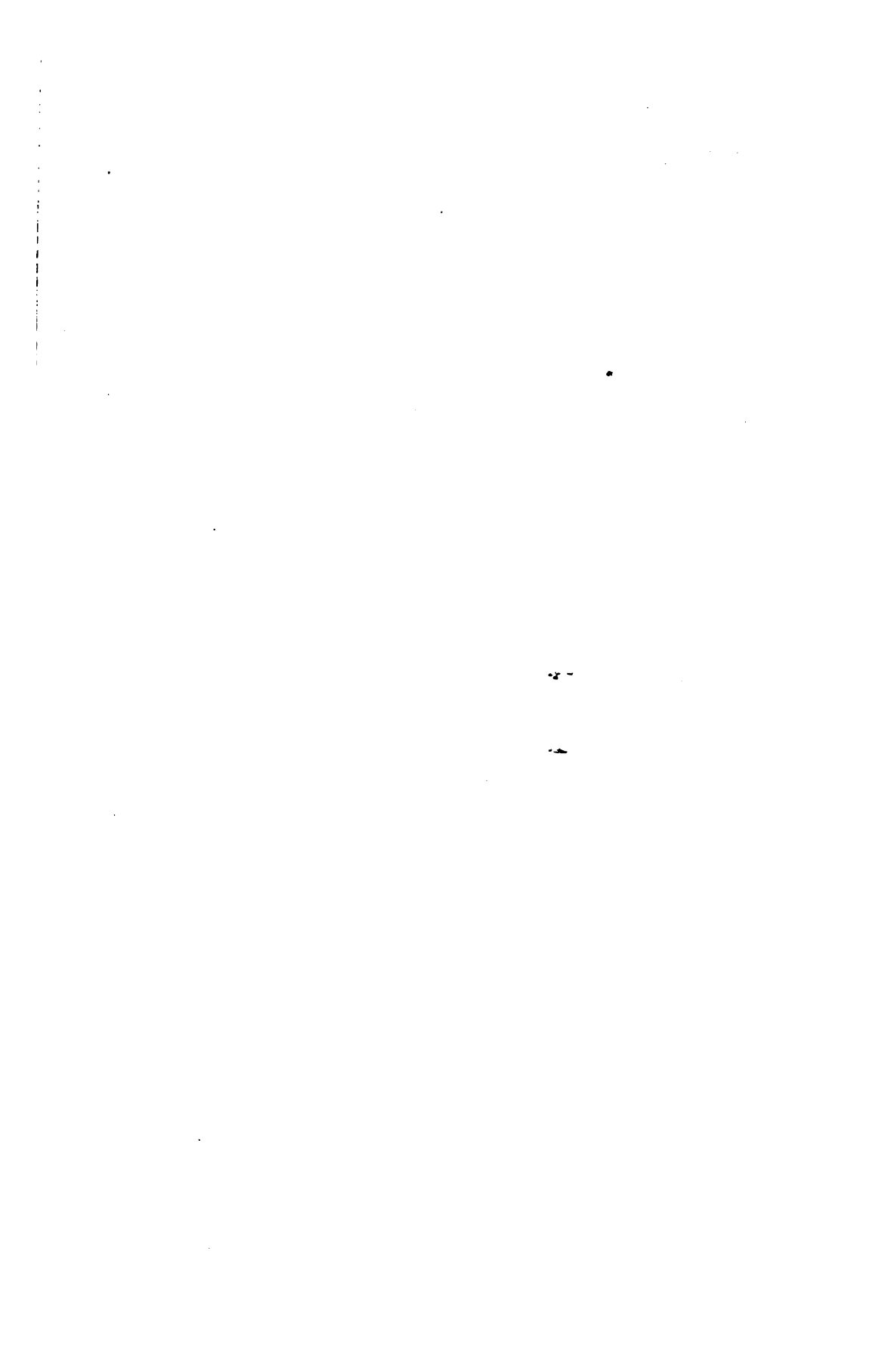
FIG. 7, $\times 40$. Surface view of a normal embryo about four weeks old. Picronitric acid, borax carmine, clove oil. The point that will interest us here is the further specialization of the cephalic lobes. The median eye tubes are still unfused, and open by separate pores, *p.e.s.* The proximal portion of the median eye nerves is seen at *p.m.n.*, connecting the median eye tubes, *m.e.s.*, with the semicircular lobes, *s.l.* The latter, which are formed by invagination of the anterior margin of the cephalic lobes, have grown inwards and backwards, so that they now lie on the dorsal surface of the same. They are seen through that part of the brain which lies over them, and which will soon give rise to the cerebral hemispheres.

The olfactory organs, *o.l.*, are now visible for the first time as oval ectodermic thickenings on the anterior margin of the cephalic lobes, at the point where the future cerebral hemispheres, the optic ganglion, and the semicircular lobes meet. The coxal sense-organs are visible as elongated transverse ridges at the median margin of the base of the appendages. From these thickenings arise the enormous ganglia of the pedal nerves and the coxal sense-organs. See my paper on the "Brain and Sense-Organs of *Limulus*."



B





EXPLANATION OF PLATE III.

FIG. 8, $\times 60$. This is a well-developed and nearly full-sized embryo of stage C-D.

It is remarkable on account of the invagination of all the thoracic appendages except the first and last pairs. The first pair are small for this stage, and hardly recognizable even in sections. The last pair are well developed, and project freely from the surface in the normal way.

The invagination of the remaining thoracic appendages is greatest on the left side. The series of low oval elevations represents the uninvaginated bases of the appendages, and the transverse slits at their summits, *w.sp.*, the openings leading into deep cavities formed by the invaginations of the outer two-thirds of the same. The dark ring around the slits represents the optical sections of the ectoderm nuclei that form the wall of the invagination. Sections of these appendages show that they do not differ materially from those shown in Pl. XI, Fig. 1.

The abdominal plate is very short and devoid of appendages. A small, deep pit is present in it, which in sections resembles the proctodaeum. Comparison with stages C and D indicates that the embryo is too young to be normally provided with a proctodaeum. It probably represents the last stages of the *telopore*. The cephalic lobes are abnormal in shape and structure. They contain two central depressed areas of thickened ectoderm, appearing as light round spots in surface views, *br.iv.*, and in section, Pl. XI, Fig. 8. The optic ganglia, *op.g.*, are partly concealed by a broad fold of ectoderm, whose free edge is directed diagonally backward, *g.f.*, Pl. XI, Figs. 8 and 8^a. This fold does not extend across the median line in front of the oesophagus.

The lateral eyes are conspicuous, but the sense-organs opposite the third thoracic appendage are not visible in surface views, although they can easily be detected in sections.

FIG. 9, $\times 60$. This embryo is between stages C and D. The anterior end of the body is narrowed, and there is a slight lateral constriction opposite the fourth pair of appendages. The latter are completely invaginated into the yolk, leaving two slit-like openings on the surface of the embryo. The boundaries of the inner ends of the invaginated appendages are not sharply defined, and in the yolk around them is a halo of degenerating nuclei apparently formed by the disintegration of its walls. The thoracic portion of the marginal fold is very faint, except at the posterior end. The whole embryo lies flush with the surrounding surfaces, instead of being deeply depressed as in Figs. 8, 10, 12.

The cephalic lobes are clearly outlined. A series of transverse sections (Pl. XI, Fig. 9) shows that the dark area bounded by the lines *cc.* and *g.f.* is covered by an amnion-like, ectodermic fold (although the middle layer is very obscure), extending backward and medianly over the cephalic lobes. Over the light inclosed area, *br.*, there is no superficial ectoderm, and the very light spots, *br.iv.*, are the same pit-like depressions of the brain seen in Figs. 8-12. The ectoderm over these depressions is covered with a layer of vertically striated, cuticular substance, looking like a layer of cilia, and similar to that seen over the surface of most developing sense-organs.

FIG. 10, $\times 60$. A large embryo, a little older than stage *C-D*. All the thoracic appendages are large. The third pair are completely invaginated, and project their whole length into the yolk. The pockets thus formed open to the exterior by two sharply circumscribed transverse slits, *p*.

The apex of the fourth appendage of the left side, *ap.*, is invaginated, and the base is expanded and folded as though vertically compressed. A section of the third pair is shown in Pl. XI, Fig. 10¹. The ectoderm is sharply defined except at the tip, *x*, where the arrangement of the nuclei indicates a proliferation of ectoderm cells into the yolk. A similar condition has been observed in other embryos. The cephalic lobes are modified in appearance by the extensive overgrowth of an amnion-like, ganglionic fold along their anterior margin. Longitudinal sections in the planes indicated by the lines 1, 2, and 3 are shown in Pl. XI, Figs. 10¹, 10², and 10³. Abnormal depressions in the region of the future cerebral hemispheres are seen at *br.iv.*, in Fig. 10, and in sections in Fig. 10².

Near the median line the brain is covered with a layer of ectoderm cells, Fig. 10³, not developed in the lateral areas, Figs. 10¹ and 10².

The lateral eyes have moved back to a point opposite the second pair of appendages. The large thoracic sense-organs are very feebly developed, if not entirely absent.

There is a conspicuous mass of cells lying in the yolk back of the tail lobe, *p.a.c.* It is formed by the concrescence of the thickened rim of the mesodermic area.

FIG. 11, $\times 60$. This embryo is similar in general appearance to that shown in Fig. 10. The thoracic appendages are, however, longer, and there are four pairs of abdominal appendages present. The distal half of the third thoracic appendage on the left side is invaginated, *th.ap.* The fourth and fifth appendages are directed forwards instead of backwards. The brain-pits, *br.iv.*, are here smaller than in Fig. 10, and pushed farther forward beneath the ganglionic fold. The whole brain and optic ganglia, *op.g.*, are depressed below the level of the ventral cord and the margin of the ganglionic fold. The ganglionic invagination, *g.iv.*, is about the same as in Fig. 10. The light area, which indicates the mouth of this slit-like depression, gradually runs into the rounded brain-pits on the median side, *br.iv.* The ganglionic fold is continued as a thickened band, without infolding, across the median anterior border of the cephalic lobes.

The lateral eyes are unusually conspicuous, and lie very nearly on a line with the chelicerae.

A large peripheral vesicle, *pr.ve.*, extends inwards almost to the centre of the egg. It is a conical cavity in the yolk, lined with a thick layer of cells, derived from an abnormal growth of the thickened margin of the mesodermic area. A well-marked depression is present in the abdominal plate that in surface views appears to mark the beginning of the proctodaeal invagination.

FIG. 12, $\times 60$, stage *C.D*.

The thoracic appendages have the character of those seen in the early phases of stage *D*, while the abdomen has the characters seen in stage *C*. The apex of the third left leg is reduced in size and deeply invaginated.

The cephalic lobes, which have run together to form a broad, unpaired thickening, are thrown to the left by a remarkable growth of the right half of the cheliceral segment. The original right chelicera has apparently divided, producing appendages *a*³⁻⁴ and *a*¹⁻². A second division followed, producing *a*³ and *a*⁴,

and a third has imperfectly divided α^1 - α^2 . The resemblance of the extra appendages to the normal chelicerae, and the fact that all the remaining appendages are in their proper positions, remove all doubts as to their identity.

It should be observed that the fourth chelicera, if this explanation is correct, is the oldest, as indeed its size and isolation suggest. This condition, however, is the reverse of what usually obtains in segmented animals. There is no distinct segmentation of the right cord corresponding to the extra appendages. But if present it could not be easily seen, as the segmentation of the nerve-cord in other parts of the embryo is very indistinct. But there is a triangular swelling of the nerve-cord, *S*, which widens forwards, and seems to include the first three appendages of the right side. A small, triangular thickening, posterior and lateral to the one just described, lies opposite the fourth chelicera.

The lateral eye of the left side is normal. But on the right are two dark areas which probably represent two right lateral eyes. It would thus appear that the right half of the cheliceral metamere has given rise by division to four more or less complete half-metameres.

FIG. 13, $\times 60$. A large embryo in stage *C*.

The first two thoracic appendages are absent on the right, causing a spiral curvature of the head toward that side. The anal plate forms a conspicuous oval protuberance, suggesting the early stages in the formation of the tail in insects and scorpions.

The most singular feature, and one not seen in any other abnormal form, was the fact that the cephalic lobes formed a continuous thickened mass of ectoderm, without distinction into right and left halves. In the median line was an enormous wedge-shaped ectodermic thickening, reinforced by an underlying layer of mesoderm, extending backward as far as the third thoracic metamere. This median thickening seems to be, in part, an exaggeration of a median post-oesophageal proliferation frequently seen in normal embryos of this age. On the right side of the cephalic lobes mesoderm and ectoderm are indistinctly separated from each other, and the former consisted of a mass of the characteristic, degenerating nuclei. In place of the first two appendages on the right, are irregular, flattened masses of loose cells containing degenerating nuclei. Nearly all the mesoderm of the right side, and especially the great masses in the appendages, showed the same kind of degenerating nuclei. None were seen on the left side.

A large area in the yolk, beneath the posterior, thoracic region, and nearly the whole surface of the embryo, especially on the right side, was strewn with innumerable, intensely red dots, that look like bacteria. But these dots are also similar in size and color to the chromatin granules in the yolk nuclei, and also to those in the nuclei of certain ectoderm cells. After a careful study of them it seems probable that the red dots will turn out to be bacteria, but we must not lose sight of the possibility that they may be chromatic granules, liberated by the rupture of degenerating nuclei. The granules appear to lead an independent existence, at least for a limited period.

FIG. 14, $\times 60$. A small embryo in stage *C.D*.

The right chelicera is absent, and the last two thoracic appendages on the right side are completely invaginated. There is a difference in direction of the first three and the last three pairs of thoracic appendages, similar to that in Fig. 22.

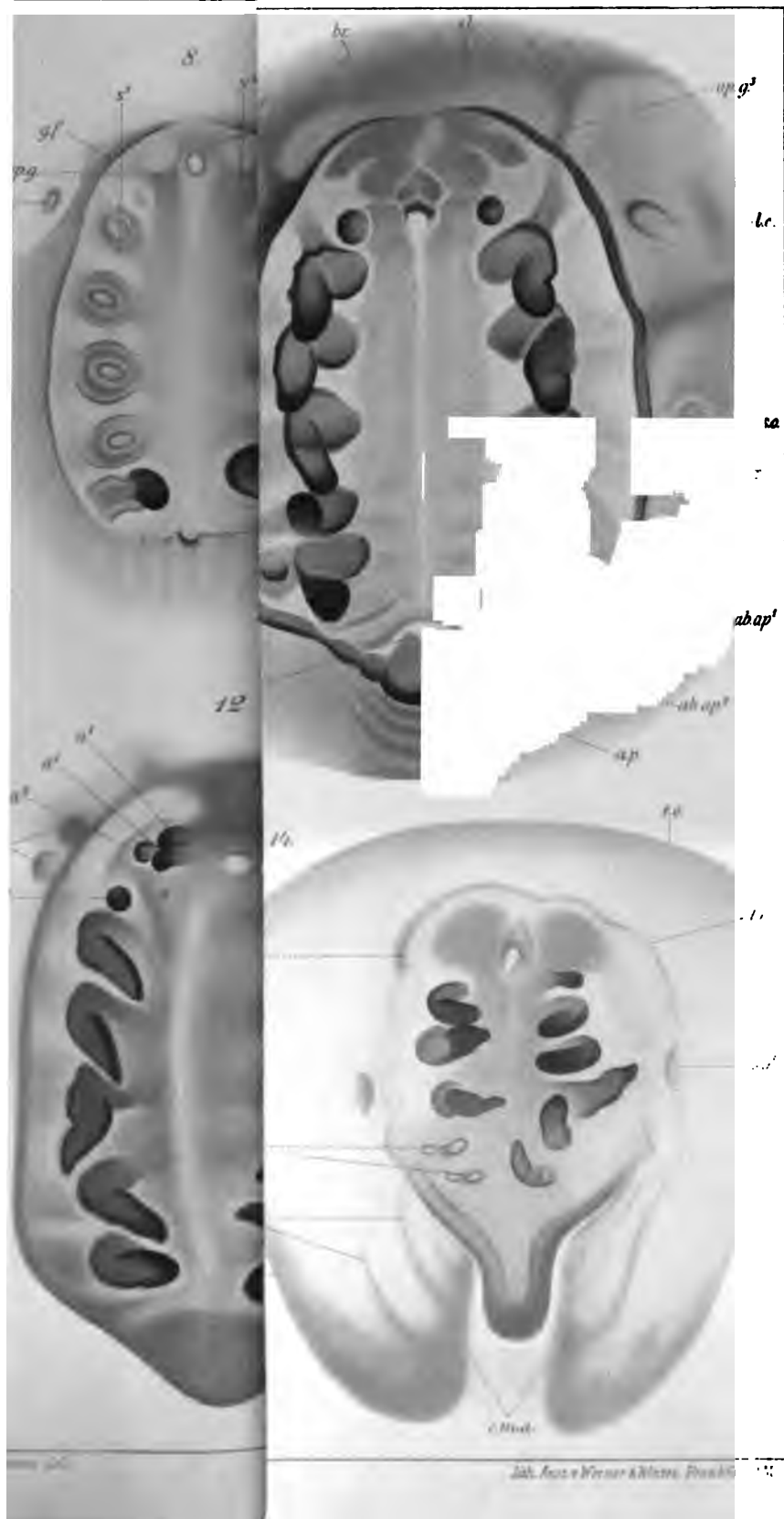
The abdominal plate forms an elongated, thick-walled lobe, the posterior half of which projects freely from the surface of the egg, a not infrequent abnormality.

The cephalic lobes are well defined, and in shape and in the numerous small, pit-like depressions of the surface suggest the early stages in the cephalic lobes of scorpions and spiders.

The thoracic portion of the marginal fold is very faint except at the anterior and posterior ends.

The posterior margins of the mesodermic area form conspicuous, triangular bands, approaching each other toward the median line. Just beneath them in the yolk are many degenerating nuclei, especially numerous just beneath the posterior median margin, where they form two elongated, parallel cords of yolk nuclei.

FIG. 15, $\times 60$. This is a remarkably large and well-developed embryo in stage *D*. It represents a type very frequently seen. It is quite normal except in its size, in the abbreviation of the abdominal plate, and in the absence of the invagination of the cephalic lobes. The whole embryo is deeply imbedded in the yolk, and with a very prominent marginal fold. There is a series of rounded ectodermic thickenings on the outer side of the base of each of the first four thoracic appendages, resembling the flabellum of the sixth pair, but being in reality the ventral ends of the dorso-ventral muscles.



EXPLANATION OF PLATE IV.

FIGS. 16, 17, (18), 19, 20, 21, (22), 23, 24, 26, 27 represent very common types of about stage C. They are often laterally compressed, as well as diminished in length by the antero-posterior compression of the head region, and by the absence of the abdomen. Except in Fig. 19, the marginal fold, *g.f.*, is very conspicuous, extending nearly around the embryo. All the parts included in this fold, *i.e.* the nervous system and appendages, are deeply depressed below the surface of the ovum. In Fig. 19, there is a difference in direction between the appendages of the anterior and posterior thoracic regions, and a wide space between the third and fourth pairs, in Fig. 23. In Fig. 16, the second and third pairs of existing appendages are partly invaginated. There is no invagination of the abdominal plate in Figs. 17 and 19, but a very conspicuous one in Figs. 16, 20, 21, and 23. The full number of thoracic appendages are present in Figs. 17, 19, 20, 22, and 23. In Fig. 16, two metameres are absent, probably the first two, although there is no positive evidence in this case as to which ones. In all the figures the cephalic lobes, and the cheliceral segment when present, are much more deeply depressed than the rest of the nervous system. This is a very common feature in this type of embryo, and is especially well shown in Fig. 16, where there is a very abrupt descent from the level of the ventral nerve-cord to that of the cephalic lobes. Compare also Figs. 62-67, etc.

Two extreme modifications of the ganglionic folds of the cephalic lobes are to be seen. One is shown in Fig. 19, where the cephalic lobes are nearly flat and naked, or as in this embryo, covered by a very thin single layer of cells, *g.f.*, advancing posteriorly and medianly over the lobes and adhering very closely to their outer surface. The other type is seen in Fig. 21, where there is a deep invagination on either side of the cephalic lobes, overarched by a very prominent, amnion-like ganglionic fold. In Figs. 17 and 23, only a small part of them is so concealed, while in Fig. 16 they are entirely exposed. In Pl. IV, Fig. 34, the whole cephalic lobes are covered by this fold.

In nearly all of these figures, the lateral eyes are very well developed for this stage, and are easily seen in surface views about opposite the chelicerae, or if the chelicerae are absent (as in Fig. 16) about opposite the position the chelicerae would have occupied had they been present. On the other hand, the so-called "dorsal organs" are either entirely absent, or so faintly developed that they cannot be detected in sections or surface views. They are shown in Fig. 22 only.

In these compressed embryos, the rim of the mesodermic area is generally very thick and distinct. In Fig. 20, the outlines of the posterior mesoblastic somite are visible up to the margin, and the manner in which they grow toward the median line, and unite there to form the post-anal cloud of cells, is clearly shown.

In most embryos of this type, the outlines of the somites are not preserved, but the posterior portion of the thickened rim of the mesodermic area, and the part that has concresced in the posterior median line are all the more distinct. One of these forms, seen as an opaque object and illustrating the extreme development of this character, is shown in Pl. VI, Fig. 63. An important point to be observed here is that the rim of the mesodermic area is so abnormally large, that it appears as a white and prominent blade, resembling in shape and position the so-called concrescing margin of the blastopore in sharks and reptiles.

The prominent mesoblastic rims of this type of embryo are subject to local vesicular enlargements, which are usually filled with a clear fluid and lined with several layers of rounded cells derived from the mesoderm. The round cells present all stages of degeneration.

The individual characteristics of these embryos are as follows :

FIG. 16, $\times 60$, sectioned. Two thoracic metameres are absent, probably the first two. Last three pairs more or less invaginated at apex. Embryo somewhat elevated as a whole, but depressed in centre, and surrounded by a high, thick, marginal fold. Abdomen absent. Cephalic lobes, sharply depressed below level of rest of nervous system, forming a steep descent between the first two appendages. Two deep oval invaginations on the lateral margins of the cephalic lobes.

There is a slight depression at the summit of the elevation between the first two thoracic appendages, from which arises by inward proliferation, a great mass of degenerating cells, which give this area its dark color. The region of most active proliferation seems to extend a short distance along the median line, the crowd of cells thus produced mingling anteriorly with those about the oesophagus, and diminishing posteriorly till they disappear about opposite the anterior margin of the second pair of appendages.

The mesodermic area is extensive anteriorly, but without a conspicuous rim, which was too remote from the body of embryo to represent in the figure. Two broad masses of mesoderm radiate from the head of embryo to the rim of the mesodermic area. Posteriorly the mesodermic rim is well marked and notched in the median line, where there is a small ectodermic elevation, *p. a. c.*, continuous with a great mass of underlying yolk cells. The latter are also continuous with the great cloud of cells arising from the deep oval invagination, *t. p.*, in the anal plate. There is a small marginal vesicle on the right side, *m. v.* No trace of segmentation in the mesodermic area.

FIG. 17, $\times 60$, sectioned. Embryo short and rather broad. Abdomen absent. Marked difference in size of the first, the following three, and the last two, pairs of appendages. Cephalic lobes broad and disproportionately large. Large oval depressions, *br. iv.*, on sides of lobes partly covered by an overhanging ganglionic fold. Marginal fold conspicuous posteriorly, where it extends across the median line, forming a very prominent spindle-shaped enlargement. No marked concrescence of the posterior margin of the mesodermic area.

FIG. 18, $\times 60$, sectioned. The body of this embryo lies well below the surface and is much compressed laterally, so that the nerve-cord forms a single, median, roof-like ridge, not adequately represented in surface views. The cephalic lobes are absent, but there is a small median invagination, probably representing the oesophagus.

There is a very large tail lobe, projecting upward and forward, and entirely separated from the body except at its posterior end. Its interior is filled with yolk. On either side are seen the median portions of two great marginal vesicles, *m. v.* The outer margins of the vesicles coincide with the peripheral margin of the mesodermic area. In the depressions beneath the legs and the tail lobe, on the nerve-cord and elsewhere, the surface of the embryo was covered with clusters of the intensely red dots that look so much like bacteria.

Beneath the base of the tail lobe is a conical invagination best seen in sections, directed diagonally backward into the yolk. From its posterior end, streamers of closely packed cells extend into the yolk. Around these streamers are many free nuclei resembling yolk cells.

Back of the tail lobe is the usual ectodermic thickening, *p.a.c.*, and beneath it is the usual post-anal cloud of mesoderm, formed by the conrescence of the margin of the mesodermic areas.

FIG. 19, $\times 60$, not sectioned. Embryo not depressed. Thoracic appendages show a common, but abnormal mode of growth. Abdomen absent. Cephalic lobes slightly convex, with very thin ectodermic fold, probably consisting of a single layer of cells growing over their lateral portions. Marginal fold knotted and conspicuous posteriorly, but not continuous across the median line. No invagination of anal plate. Mouth very long and narrow. Mesodermic area, comparatively small, circular, and with conspicuous rim, *m.a.*, especially at sides. No conspicuous mass of cells due to conrescence of mesodermic rim back of anal plate.

FIG. 20, $\times 60$, sectioned. Embryo short and narrow and deeply depressed. Marginal fold sharply defined. Cephalic lobes shortened and partly concealed by a broad overhanging ganglionic fold.

Abdominal plate without appendages and sharply infolded to form a very deep oval invagination, from the walls of which arises a cloud of degenerating yolk cells.

The mesodermic area is extensive. Its rim is well defined, and posteriorly shows very beautifully its mode of conrescing. In this case the outlines of the posterior, mesoblastic segments are very clearly shown.

FIG. 21, $\times 60$, sectioned. Chelicerae and abdomen absent. Large open depression on margin of cephalic lobes, bounded on the sides by a prominent, overhanging lip. Mouth very large, with rostrum-like projection in front. Body of embryo depressed and surrounded by prominent, knotted, marginal fold, which is discontinuous posteriorly.

A deep and broad invagination of the anal plate has carried the last pair of thoracic appendages inwards till they project from its sides. A conspicuous elevation back of anal plate, *p.a.c.*, due to the conrescence of the posterior margin of the mesodermic area. The lateral and anterior portions of the rim are not represented.

FIG. 22, $\times 60$. A very common form of embryo in stage *D*. Not sectioned. Differs from the normal in its shortened, compact shape, and in being deeply sunken in the yolk. It was outlined to show a rather frequent modification of the direction in which the thoracic appendages grow, the anterior pairs pointing backwards and laterally and the posterior ones forward and inwards. It is interesting because it occurs frequently, but especially because it is between the third and fourth thoracic metameres that transverse division sometimes takes place. The difference in the disposition of the appendages recalls a very similar one that obtains between the mouth parts and the walking appendages of insect embryos.

FIG. 23, $\times 60$, not sectioned. Embryo narrow and depressed. Appendages normal except in the separation of third and fourth pairs, a rather frequent occurrence, that seems to have some connection with the transverse fission that often occurs here, and with the difference in direction between the appendages in front of and behind this space. Two pairs of abdominal appendages present, but they are carried inwards by the invagination of the anal plate, so that they project from the sides of the cavity. Marginal fold prominent, especially posteriorly, and continuous anteriorly with a well-defined fold overhanging the invagination on the sides of the cephalic lobes. Mesodermic area not conspicuous or well defined.

FIG. 24, $\times 60$, sectioned. In this embryo the cephalic lobes and first two thoracic segments have disappeared. The marginal folds have closed in front of the third pair of appendages, and the nerve-cord terminates abruptly just back of them. The abdomen is absent, and the marginal folds are contracted so as to extend across the median line just behind the sixth pair of thoracic appendages. In the median line, the fold is interrupted by a deep, thick-walled invagination with an oblong lumen.

The invagination dips deeply into the yolk, and from its thick walls arise numerous nuclei which are seen scattered about in the neighboring yolk. The yolk nuclei are most numerous back of the invagination. An ectodermic thickening forms the broad, dark, post-anal band seen in surface views, *p.a.c.* At its posterior end, the band becomes continuous with the thickened rim of the mesoblastic area. The post-anal cloud of yolk cells is formed in part by cells that have migrated from the walls of the invagination, and in part by those arising from the thickened mesodermic rims, which have concresced along that line.

A large marginal vesicle, *m.v.*, is seen in the right anterior margin of the mesodermic area.

FIG. 25, $\times 60$, sectioned. A remarkable embryo in stage *C*, in which the cephalic lobes are reduced to a flat circular plate, slightly depressed, so that it is surrounded on all sides by a vertical wall. Near the centre of the disc, which is separated by a considerable distance from the remainder of the embryo, is a small pit representing the oesophagus, and in the yolk below the disc is a great, irregular mass of cells, with numerous pseudopodia-like streamers of cells, extending still deeper into the yolk.

The body of the embryo, which consists of three appendage-bearing segments, is bent into the yolk at the posterior end. Back of this abbreviated trunk is a broad depression bounded on either side by steep walls, which gradually shallow posteriorly to the surface of the ovum.

FIG. 26, $\times 60$, sectioned. A very compact embryo of stage *C* and *D*. All the ectodermic layers are very thick, and the mesodermic area is constricted so that its peripheral margin lies close around the body of the embryo. The greater part of the mesoderm forms two thick bands on either side of the body, close to the median line.

The cephalic lobes are completely covered by a hood-like fold extending back almost to the second pair of thoracic appendages. At the bottom of the cephalic cavity is a small pit, the histological character of whose walls indicates that it is the oesophagus.

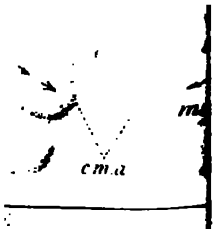
Surface of embryo covered with bacteria.

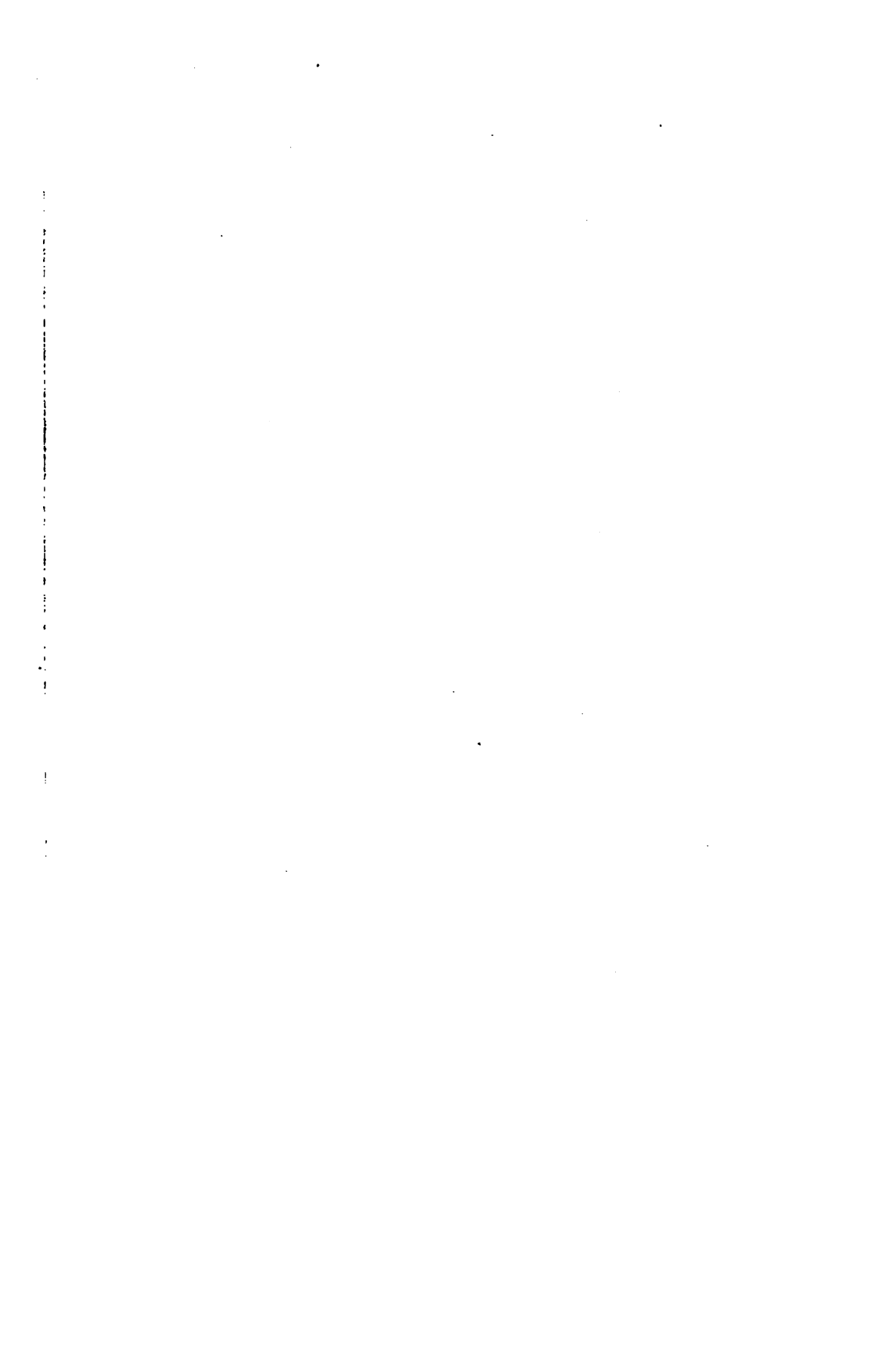
FIG. 27, $\times 60$, sectioned. Very much shortened embryo in stage *C*.

The rudimentary cephalic lobes are covered by a backwardly directed fold, and only three pairs of appendages, probably the fourth, fifth, and sixth, are represented. What looked in surface views like an anus was present, but its nature could not be determined in sections. Such embryos as those in Figs. 26 and 27 are rather common, and possibly they were seen by Dohrn and Osborn, and gave rise to the statement that *Limulus* passes through a nauplius stage.

FIG. 28, $\times 33$, not sectioned. An embryo in stage *C*.

It consists of an abdominal plate and four metameres, probably representing the last three thoracic and first abdominal ones. The appendages are separated by a very wide space, over which the neuromeres (?) and mesoblastic somites extend as long, narrow bands.





EXPLANATION OF PLATE V.

FIG. 29, $\times 60$. Embryo in about stage *E*.

The most striking features are : (1) a diffuse atrophy of the left side, resulting in the complete disappearance of the left abdominal appendages and neuromeres, and the reduction in size and absence of surface details in the left thoracic appendages; and (2) the *hour-glass form* due to a diffuse, transverse atrophy of both halves along the fourth thoracic segment. When examined more carefully, it is seen that the three anterior thoracic appendages on the right side are nearly normal; the second and third being perhaps a little stouter than usual, but still showing all the characteristic details of this stage. The fourth right appendage is reduced to a broad, low elevation, probably representing the basal joints of the same, the rest of the appendage being reduced to a very small, medianly directed protuberance springing from an oval depression. The next appendage is larger than the fourth, but smaller and less perfect than the sixth; and that is smaller and less perfect than it should be. The last two look as though made of wax that had been warmed on the surface, thus melting off sharp angles and other details.

The right side of the abdomen is apparently perfect.

On the left half of the thorax the chelicera is thrown outwards, but is otherwise normal; but the second and third appendages are strikingly smooth and rounded on the tips, as though the surface details had been melted off. The fourth is a minute papilla, and the fifth and sixth are small, rounded, unjointed elevations.

The whole left half of the abdomen is absent, except a trace of the marginal fold.

The cephalic lobes are somewhat distorted, and the semicircular lobe is apparently interrupted in the median line.

The median ocellus and the nerves extending to it are very distinct.

The ventral nerve-cord is not clearly divisible into right and left halves. Back of the third pair of thoracic appendages, it narrows, and finally the right half only extends beyond the rudimentary fourth pair of appendages into the abdomen. In another specimen similar to this, it is very clear that the right half of the cord only is represented on the posterior part of the thorax and abdomen.

The marginal fold is deeply constricted opposite the fourth thoracic segment. The right lateral eye and "dorsal organ" lie beyond the limits of the figure, thus preserving their normal position in reference to the body of the embryo, but not in reference to the marginal fold. The left eye and dorsal organ could not be seen, and were probably much reduced, if not entirely absent.

FIG. 30, $\times 60$, not sectioned. Embryo with two thoracic appendages and the corresponding neuromeres of the left side absent. The missing appendages appear to be the second and third, as the next three correspond to the last three of the opposite side. It should be observed that the second and third appendages of the right side are unusually large, as is the case with the appendages on the corresponding segments in Figs. 31 and 32. There appeared to be four abdominal appendages on the left and only two on the right. There is a very large peripheral vesicle on the right side, *p.v.*

FIG. 31, $\times 60$, not sectioned. Embryo with the last three thoracic appendages on the left side absent. The left half of the abdomen is also absent. The

remaining half, which is provided with two distinct appendages, is thrown sharply to the left, so that its long axis is at right angles to the rest of the body.

All the thoracic appendages are present on the right; but the second and third are very large, and the fifth and sixth correspondingly small. On the left, three appendages are absent, probably the last three of the series, as the most anterior one seems to represent the chelicera.

FIG. 32, $\times 60$, not sectioned. On the left side the second and third thoracic appendages, as in the preceding figures, are unusually large, and the last three thoracic appendages are abnormally small. On the right the chelicera is absent, and the last three appendages, of which one is entirely absent, are very small. The right half of the abdomen is absent, but on the left three appendages and their corresponding neuromeres are present. What is left of the abdomen is thrown to the right, and its end covered with a large hood-like overgrowth of the marginal fold.

FIG. 33, $\times 60$. Small embryo in stage C.

Cephalic lobes are very small and partly concealed by the ganglionic fold, which nearly reaches to the mouth. Three well-formed appendages, fourth, fifth, and sixth (?), are present on the left. On the right the corresponding appendages are invaginated, forming three shallow pits, *i.a.* The abdomen and the anterior part of the thorax are absent.

The outline of the mesodermic area is visible, showing its thickened rim, *ma.* Its constricted posterior portions form the usual post-anal cloud of cells, *p.a.c.*

FIG. 34, $\times 60$, not sectioned. A much reduced embryo in stage C.

The head is turned toward the left, although that side is better developed than the right. The cephalic lobes are covered by a distinct, amnion-like fold, that extends back to the first remaining appendage. Through the fold may be seen the reduced oesophagus. Two appendages are present on the left, and corresponding to them on the right, a shallow pit and small papilla. Back of these appendages is a large unpaired one, probably formed by the fusion of the appendages of the sixth pair. Back of this is a deep furrow, flanked on either side by a rounded elevation.

On either side, just within the limits of the mesodermic area, are two large marginal vesicles.

FIG. 35, $\times 60$, not sectioned. A much distorted and abbreviated embryo.

The cephalic lobes are broad, and contain a good-sized oesophagus. The remainder of the embryo is bent sharply to the right. This is due to the presence of three distinct appendages on the left side, and only one (the second?) on the right. The tail end of the embryo is invaginated, and covered by a small, overgrowing fold. The margin of the mesodermic area is clearly defined, and plainly shows the notch, due to conrescence of its posterior margin. At the anterior end of the embryo is a dark band of yolk cells (?) extending forward to the anterior border of the mesodermic area.

FIG. 36, $\times 42$. Embryo in somewhat older stage than Fig. 29.

The whole right half is practically normal. The most characteristic feature of the embryo is the absence of most of the left half of the thorax; although the left half of the cephalic lobes, and of the abdomen, is nearly normal.

The whole embryo is somewhat shortened. The appendages of the right side, and the right ganglionic fold, extends backwards over the outer surface of the

brain and optic ganglion farther than usual. Otherwise the right side appears to be normal.

The left sixth thoracic appendage is a small, three-jointed organ, showing distinctly the characteristic flabellum near its lateral margin. The fifth is a mere papilla, and the third and fourth are entirely absent. The second is relatively large, and seems to be partially invaginated at the tip. The left chelicera is absent. In the left half of the abdomen the appendages are partly fused, forming a great three-lobed mass.

It is, therefore, plain that while the left half of the thorax is greatly reduced, as a whole, it shows in addition a *diffuse transverse atrophy with its line of greatest intensity between the third and fourth appendages, and diminishing gradually in front and back of this line*. We thus have a case of *hour-glass atrophy, confined to the left half of the body*. (Compare Fig. 29.)

The nervous system is not sharply outlined. The parts of the cephalic lobes are run together, and a great dark fold extends diagonally backwards over the right half. The left is much smaller, and has been carried backward by the contraction of the left side. The oesophagus and mouth are small and inconspicuous. It is not clear from surface views whether a part of the left nerve-cord is absent or not.

The lateral eyes and dorsal organs were in their normal positions on the right side, but could not be seen on the left.

The marginal fold is normal on the right. On the left it disappears near the optic ganglion. There is a large, laterally directed fold opposite the left sixth appendage that consists, in part at least, of the marginal fold. In the abdomen the left marginal fold is normal, and it meets the fold just described at a sharp angle, but does not appear to be continuous with it. Between the two folds is a very deep triangular depression. This condition of the marginal fold is the usual one when there has been atrophy of the anterior quarter of the same side.

In the anal region the marginal fold is greatly thickened, forming a conspicuous U-shaped boundary to the posterior part of a deep depression.

FIG. 37, $\times 40$. This embryo is, in part, in an advanced stage of development, corresponding to that seen in embryos about ready to hatch. There are only a very few markedly abnormal embryos found in this late stage.

The right side is nearly perfect and normal. The ganglionic fold over the right half of the brain is large, as in the preceding figure. The right nerve-cord, which was very well developed and plainly outlined, is bent sharply at the junction of the thorax and abdomen. This bend, as seen in the drawing, is due, to a small extent only, to the foreshortening produced by looking down on the upturned abdomen. Almost the entire left half of the cephalic lobes are absent, but the left nerve-cord, as far as the beginning of the abdomen, is present in its normal condition. All the left thoracic appendages have disappeared completely, leaving no trace behind except in the third segment, where there is a shallow depression, seen edgewise in the figure, with a small papilla projecting from its centre. This papilla probably represents the last trace of the third thoracic appendage. The left nerve-cord seems to terminate abruptly at the posterior end of the abdomen, without uniting with its abdominal part. The anterior end of the latter turns off sharply to the left, toward a large, dark, conical elevation. To the left of it is a short, conical projection, with its apex directed forwards.

The embryo is probably in a state of incomplete longitudinal fission, or a double embryo, similar to those in Pl. IX, Fig. 98. The two problematical appendages, x and y , would then represent the medianly fused, posterior thoracic appendages of a second embryo, whose axis is in the line B . The two embryos possess an abdominal nerve-cord in common. The abdominal appendages on the left side of embryo B are absent (its right abdominal half was not formed at all according to this supposition), and in embryo A the thoracic appendages failed to develop after the longitudinal fission of the embryo.

This explanation, requires us to assume nothing more than what we know takes place in the double embryos described in plates IX and X. If it is correct, this embryo, although resembling that in Fig. 36, owes its present condition to a totally different sequence of events.

FIG. 38, $\times 33$, unsectioned. A well-advanced embryo, the right half of which is complete and perfectly normal, except in its slight curvature to the left. Of the left half nothing remains but a dark band of inner-layer cells and a small posterior appendage situated in a rather deep depression. The long axis of the mouth is rotated nearly 45° , so that the apex of the rostrum is thrown toward the left.

EXPLANATION OF PLATE VI.

FIGS. 39-49 illustrate the more important phases in the formation of the inverted V-shaped embryos. These embryos are formed by the fusion in the median line of the corresponding right and left organs of each metamere. The organs nearest the median line are the first to unite, forming in that way an unpaired organ, having the characteristic features of each member of the pair. The unpaired organ thus formed then decreases in size, and finally disappears. In its place the organs next to it, on the same metamere, unite, and in turn degenerate; and so on till the whole metamere has disappeared. The process seems to begin in every case in the most anterior metameres; and in the most typical cases, as soon as the first unpaired organ, formed in, say, the first thoracic metamere, has disappeared, the same organ is found unpaired in the second metamere; and by the time that has disappeared the unpaired condition of that organ obtains in the next following metamere, and so on, till every paired organ has become median and unpaired, and then disappeared. In the last phase of the process, if realized in full, there would be nothing left of the embryo but a single unpaired organ, situated at what was the posterior end of the body, and formed by the median fusion of the most laterally situated paired organ of the last metamere.

Such a condition has not been observed, the nearest approach to it being an embryo of which nothing remained but the mesodermic area and a posterior unpaired process, representing either the last thoracic appendage or the tail lobe.

In very rare cases one of the posterior pair of appendages may fuse in the median line, while there is no indication of fusion in front of that point. But in such cases there is no evidence of a progressive median fusion and degeneration extending toward the anterior end.

There is another exception to the median fusion and progressive antero-posterior degeneration seen in the hour-glass embryos shown in Pl. IV.

FIG. 39, $\times 60$, not sectioned. This embryo is instructive, as it is apparently in the early stages of median fusion. The cephalic lobes are reduced to a thin, circular disc, showing no trace of separate optic ganglia and cerebral hemispheres. The oesophagus is a shallow pit in the centre of the disc, and the cheliceral segment has disappeared entirely. The appendages of the fifth and sixth thoracic segments are nearly normal in position, but in passing forward from this point the appendages decrease in size and approach more and more the median line, till in the second segment they have nearly united with each other. The nerve-cords terminate back of either the third or the fourth pair of appendages, but as this embryo was not sectioned that could not be determined with certainty.

The end of the left third appendage is invaginated. The abdomen is normal except for the rather prominent anal plate.

FIG. 40, $\times 60$. This is a very unusual form, and the only one of its kind observed. Median fusion has taken place at both ends. The dorsal organs are very conspicuous, and as they always lie opposite the fourth pair of appendages we can see that the following changes have taken place:

The cephalic lobes, oesophagus, and first thoracic neuromere have disappeared. The appendages of the second pair have fused completely, and those of the third pair have approached each other, preparatory to the same change. The fourth pair is nearly normal. The fifth appendage on the right is invaginated for half its

length. The appendages of the sixth pair are short and thick, and have fused with each other except at their tips. Just in front of them is a large, deep pit, *i.v.* I do not know of any explanation of the presence of such an invagination at this point, unless it may be regarded as an invaginated tail lobe or a telopore, carried forward to its present position before the fusion of the last pair of thoracic appendages.

Every trace of the abdomen is absent, something that is very unusual, for it is a noteworthy fact that in this class of embryos the abdomen usually remains nearly normal, however profound may be the changes that have affected the anterior part of the embryo.

FIG. 41, $\times 60$, sectioned. In this embryo the cephalic lobes and first two thoracic segments have disappeared, apparently after median fusion. The primitive position of the cephalic lobes is indicated by a conical mass of degenerating cells projecting into the yolk, and seen in profile on the edge of the egg. From the apex of the mass, an irregular train of the same kind of cells, lying deep in the yolk, extends backwards to the surface at the anterior end of the embryo. These cells probably represent the last remnant of the degenerating oesophagus and anterior portion of the embryo.

The right appendage of the third thoracic segment is absent, and the fifth pair is invaginated. Three depressions along the median line are indicated by white areas.

All the parts of the embryo appear very dark, owing to the unusual thickness of the cell layers, and the large amount of staining fluid the cells have absorbed.

FIG. 42, $\times 60$, sectioned. This is a rather common form. The cephalic lobes, oesophagus, and first two thoracic metameres are absent. The appendages of the first pair are fused nearly to their tips, those of the fourth pair are fused at the base only. The two nerve-cords fuse with each other just in front of the fifth pair of appendages, and the single median cord thus produced terminates abruptly just back of the base of the fourth pair. Within the fused bases of the appendages of the fourth segment is a large mass of cells that looks like the remnant of the neuromere of this segment, but forced to assume a spherical form by the fusion of the appendages. It is connected posteriorly with the rest of the nerve-cord by a narrow chain of cells. In front of the fused appendage is merely a thin layer of ectoderm and mesoderm, every trace of the nerve-cord being absent.

Immediately in front of the fused appendages of the third thoracic segment is a short, flattened tube, directed diagonally forward into the yolk. It is surrounded by a thin layer of mesoderm, and opens outwards by a transverse opening between the marginal fold and the anterior edge of the unpaired appendage. It has all the appearance of an oesophagus, and probably is one, but of course it is entirely out of place here.

There is a slight asymmetry of the abdomen, due to the absence of one appendage on the left side, but otherwise the embryo back of the fifth pair of thoracic appendages is quite normal.

FIG. 43, $\times 60$. This embryo is similar to that in Fig. 49. The original position of the cephalic lobes is indicated by a shallow, saucer-shaped, ectodermic thickening, *c.l.* In the figure, owing to the curvature of the surface of the ovum, it is seen edgewise, and only the thickened posterior rim is shown. It is connected with the remaining part of the embryo by a broad train of *yolk cells*, lying close to the surface. They appear in the figure as a broad, faint band, *y.c.* The third

pair of appendages are fused at their bases, and back of them terminates the ventral nerve-cord.

The abdomen is smooth and flat, showing no trace of appendages.

The limits of the mesodermic area are clearly defined by the usual thickened rim. The mesodermic area back of the tail lobe is darker, and its posterior margin, owing to incomplete concrescence, is deeply notched.

FIG. 44, $\times 60$. In this embryo the cephalic lobes and the cheliceral segment have disappeared, leaving some distance in front of the embryo a large irregular patch of cells, continuous right and left with the mesodermic rim. In the figure it is seen in profile on the edge of the egg, *c.l.*

The appendages of the second post-oral segment have fused with each other, and a similar fusion of appendages has taken place in the third segment. The smaller size of the anterior median appendages indicates that it was formed previous to that of the following segment, and has already undergone some degeneration.

The marginal fold, *m.f.*, extends *anteriorly* between the fused appendages of the second and those of the third segment. It usually appears to contract with the atrophy of the anterior end of the body, so that it closely encircles all the remaining appendages. If that really occurred here, the fold must have in some way passed around the second pair of appendages, a process difficult to explain. The only alternative is to suppose that a new fold was formed back of the second pair of appendages, and that the old one has disappeared or was never formed.

The nerve-cord terminates as a blunt, unpaired process, a little in front of the fourth pair of appendages. The posterior part of the thorax and the abdomen are normal, except that the apex of the latter projects freely away from the ovum, and the whole abdomen hangs over a great blister-like vesicle filled with fluid and enclosed between the blastoderm above and a thickening of the mesoderm below. There is nothing in the preparation to suggest that this condition is due to shrinkage, etc.

The outlines of two similar vesicles (or marginal vesicles) are seen in the mesodermic area on either side of the head region, *m.v.*

FIG. 45, $\times 60$, not sectioned. This embryo shows a slight reduction of the cephalic lobes and of the third and fourth thoracic appendages of the right side. In place of the fifth and sixth appendages and the abdomen, is a large median conical protuberance. Whether the latter represents the fused fifth and sixth appendages or the tail lobe could not be determined.

FIG. 46, $\times 60$, not sectioned. The cephalic lobes have disappeared, or at least together with the oesophagus are reduced to an invaginated, conical layer of cells, constituting the dark mass at the apex of the V-shaped marginal fold, *d.c.l.*

The chelicerae have fused to form a short median process, which lies in a depression that in front is partly overarched by a semicircular fold of the ectoderm.

The appendages of the second pair have fused to form a very long, slender, corkscrew-like filament. It is attached to the embryo just back of the apex of the fused chelicerae. The appendages of the third pair are fused at their bases and somewhat diminished in size. The nerve-cord terminates abruptly just back of them.

The parts of the embryo lying back of the third thoracic segment are practically normal.

FIG. 47, $\times 60$, not sectioned. The first and second thoracic segments are absent. The cephalic lobes, however, persist as a faint disc of cells without character. This case differs somewhat from the preceding ones, in that the effects of degeneration do not increase gradually from before backward, for degeneration has been greater in the first two post-oral segments than in the cephalic lobes.

The median space between the appendages of the remaining pairs gradually increases toward the posterior end.

A still further difference between this embryo and the preceding ones is seen in the fusion across the median line of the abdominal appendages, the last appendage being the smaller one.

FIG. 48, $\times 60$, sectioned. In this embryo, the sixth pair of thoracic appendages are identified by the well-developed flabella. The long median appendage is formed by the fusion of the fourth pair of the thoracic appendages, the unfused tips of the two original appendages being still visible. The first three pairs of appendages and the entire cephalic lobes have disappeared. The nerve-cord is normal and well developed from the root of the tail lobe to the base of the unpaired appendage of the fourth segment. The abdomen is small, but possesses two pairs of normal appendages. There is a very conspicuous tail lobe, the size and shape of which suggest the idea that with the diminution in size of the embryo, the marginal fold became too large, and the excess accumulated at the posterior end to form the tail lobe. *Back of the tail lobes is a small conical projection, also visible in the sections, having all the appearance of an unpaired thoracic appendage.* The presence of an appendage in this place is very remarkable, and I am unable to offer any explanation for its occurrence there.

The body of the embryo forms the floor of a deep depression bounded by the marginal fold. The embryo stained very deeply, as it is composed of dense tissue containing a large amount of chromatin. Anteriorly, the rim of the mesodermic area, which was not visible, or perhaps overlooked in the surface views, is easily seen in the sections, apparently preserving its normal size and extension for this stage. In the sections that pass a long distance in front of the present anterior end of the embryo, the mesodermic rims are seen as widely separated as in the normal embryo of this stage. In the line midway between them, instead of the nerve-cords and appendages, there is merely a thin, undifferentiated layer of ectoderm with a few scattering mesoderm cells beneath it.

FIG. 49, $\times 60$, sectioned. The cephalic lobes and first two thoracic segments are absent. No trace whatever of these organs is to be seen in sections, although there is a dark area, due to an accumulation of loose cells, where the anterior end of the embryo should be.

The marginal folds, *m.f.*, are distinct and well developed, and extend across the median line just in front of the second pair of thoracic appendages. The latter are fused with each other at their bases, and just back of them the nerve-cord terminates abruptly in a blunt, unpaired process.

The abdomen is very well developed, and terminates in a broad tail lobe that projects upwards and forwards, thus overarched the posterior part of the abdomen.

Such a prominent tail lobe, although occasionally seen, is not a common form of abnormality. It is suggestive of the prominent tail fold in insects and crustacea, but in this peculiar case recalls that seen in scorpion embryos.

59.



45

m. or. l.

m. a.

ye



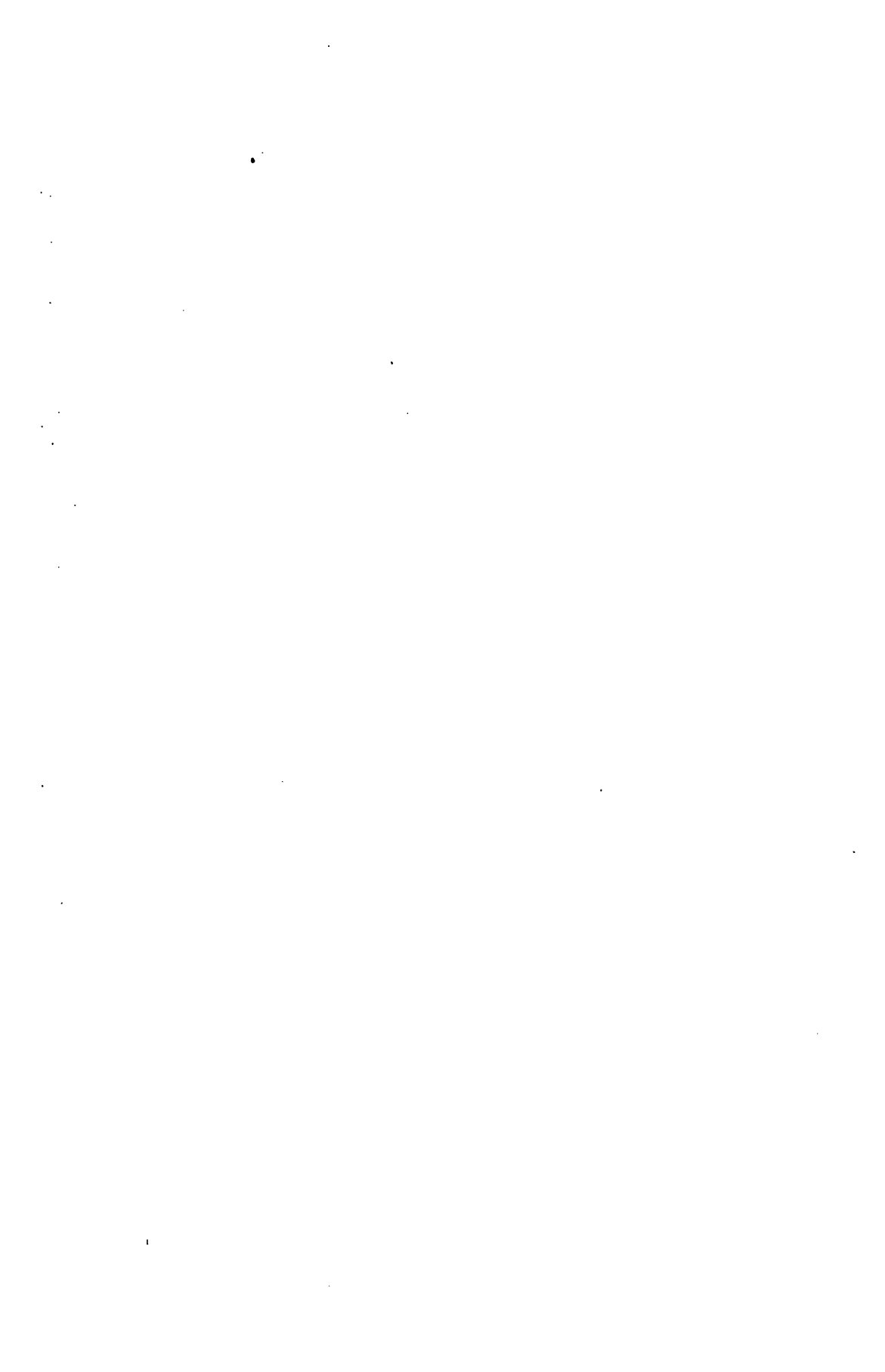
m. l.

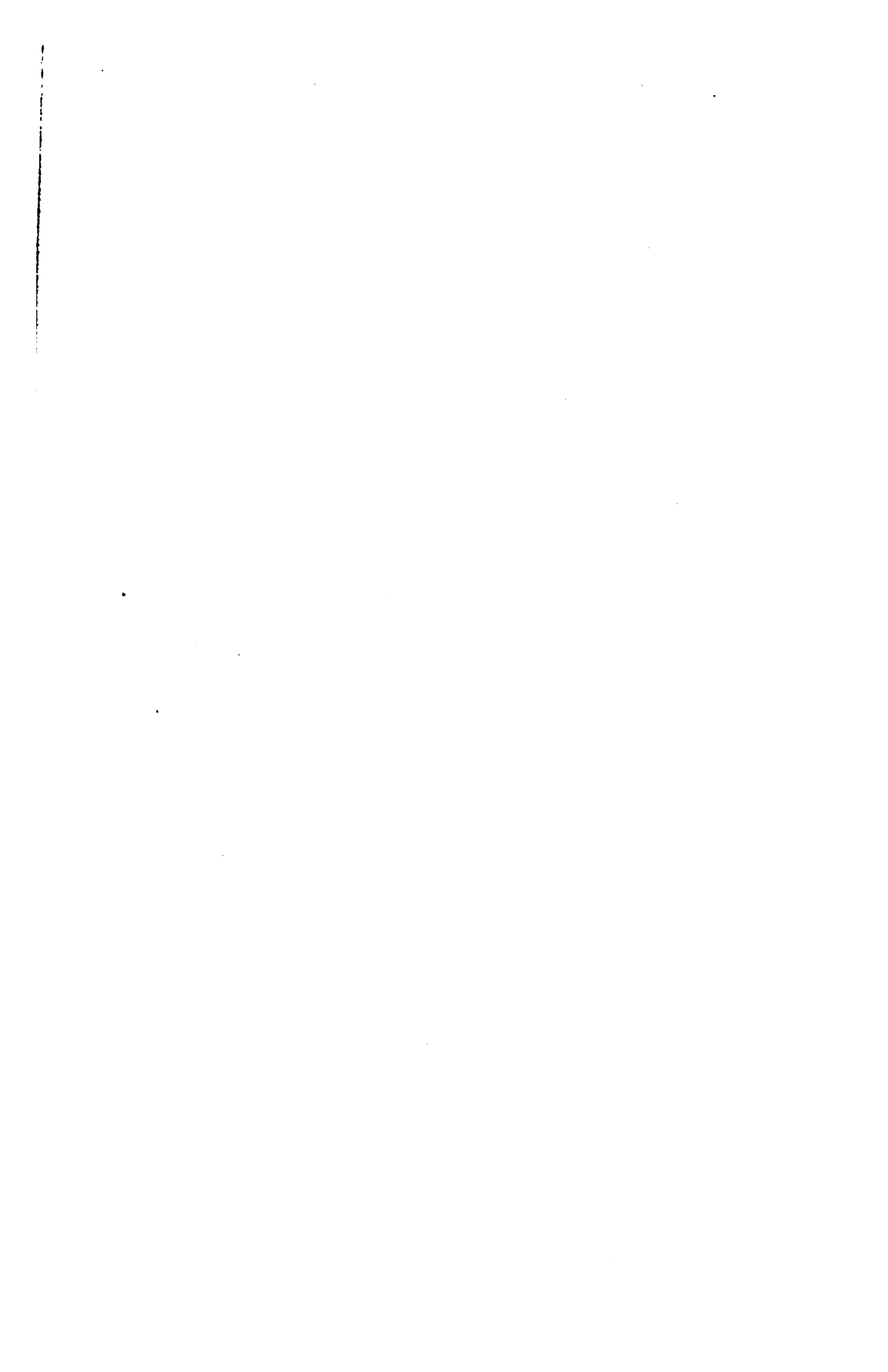
cap. a.

49

m. a.







EXPLANATION OF PLATE VI.

FIGS. 39-49 illustrate the more important phases in the formation of the inverted V-shaped embryos. These embryos are formed by the fusion in the median line of the corresponding right and left organs of each metamere. The organs nearest the median line are the first to unite, forming in that way an unpaired organ, having the characteristic features of each member of the pair. The unpaired organ thus formed then decreases in size, and finally disappears. In its place the organs next to it, on the same metamere, unite, and in turn degenerate; and so on till the whole metamere has disappeared. The process seems to begin in every case in the most anterior metameres; and in the most typical cases, as soon as the first unpaired organ, formed in, say, the first thoracic metamere, has disappeared, the same organ is found unpaired in the second metamere; and by the time that has disappeared the unpaired condition of that organ obtains in the next following metamere, and so on, till every paired organ has become median and unpaired, and then disappeared. In the last phase of the process, if realized in full, there would be nothing left of the embryo but a single unpaired organ, situated at what was the posterior end of the body, and formed by the median fusion of the most laterally situated paired organ of the last metamere.

Such a condition has not been observed, the nearest approach to it being an embryo of which nothing remained but the mesodermic area and a posterior unpaired process, representing either the last thoracic appendage or the tail lobe.

In very rare cases one of the posterior pair of appendages may fuse in the median line, while there is no indication of fusion in front of that point. But in such cases there is no evidence of a progressive median fusion and degeneration extending toward the anterior end.

There is another exception to the median fusion and progressive antero-posterior degeneration seen in the hour-glass embryos shown in Pl. IV.

FIG. 39, $\times 60$, not sectioned. This embryo is instructive, as it is apparently in the early stages of median fusion. The cephalic lobes are reduced to a thin, circular disc, showing no trace of separate optic ganglia and cerebral hemispheres. The oesophagus is a shallow pit in the centre of the disc, and the cheliceral segment has disappeared entirely. The appendages of the fifth and sixth thoracic segments are nearly normal in position, but in passing forward from this point the appendages decrease in size and approach more and more the median line, till in the second segment they have nearly united with each other. The nerve-cords terminate back of either the third or the fourth pair of appendages, but as this embryo was not sectioned that could not be determined with certainty.

The end of the left third appendage is invaginated. The abdomen is normal except for the rather prominent anal plate.

FIG. 40, $\times 60$. This is a very unusual form, and the only one of its kind observed. Median fusion has taken place at both ends. The dorsal organs are very conspicuous, and as they always lie opposite the fourth pair of appendages we can see that the following changes have taken place:

The cephalic lobes, oesophagus, and first thoracic neuromere have disappeared. The appendages of the second pair have fused completely, and those of the third pair have approached each other, preparatory to the same change. The fourth pair is nearly normal. The fifth appendage on the right is invaginated for half its

EXPLANATION OF PLATE VII.

FIG. 50, $\times 60$. This embryo evidently represents an extreme case of median fusion, but differs from those on the preceding plate in that there are no indications of that V-shaped arrangement of paired organs usually seen when a progressive antero-posterior degeneration has followed the median fusion. The cephalic lobes, oesophagus, and nervous system are entirely absent. Nothing remains of the body but an oblong elevation of thickened ectoderm with three median appendages arranged along its summit. Behind the third median appendage was a partly invaginated plug of cells, *t.p.*, that may be the remnant of the telopore. Besides this there was nothing to indicate to what segment these fused appendages belonged, or which was the anterior and which the posterior end of the body. The mesodermic area was slightly raised and was pentagonal in outline, with a thickened rim.

FIG. 51, $\times 60$. A large embryo in stage C showing a distinct transverse constriction between the third and fourth thoracic segments.

The anterior part of the embryo is normal, except in the absence of the chelicerae.

The posterior portion is infolded between the appendages of the sixth thoracic segment to form a deep, circular cavity. The sixth pair of legs, identified by the flabella on their outer margin, have been carried inwards by the infolding, till they project toward each other from its lateral walls.

FIG. 52, $\times 60$, sectioned. An hour-glass embryo in stage C.

The cephalic lobes are deeply depressed, and nearly covered by two lateral folds. The chelicerae are absent, the next two appendages are brought closely together, and the third pair completely fused to form a thick median appendage, *ap*³, extending backwards.

These three appendages project from a circular depression, bounded on all sides by a thickened margin, which anteriorly forms the folds overlapping the cephalic lobes.

The fourth pair of appendages are also fused, *ap*⁴, and the fifth are either absent, or fused and invaginated to form the pit just back of the base of the preceding pair, *ap*⁵.

The sixth pair are small and devoid of flabella, but are nearly normal in shape and position.

The posterior part of the thorax and a part of the abdomen are nearly surrounded by a marginal fold, well developed posteriorly, but not extending across the median line in front of the fused appendages of the fourth segment.

FIG. 53, $\times 60$, sectioned. A remarkable embryo in stage C that has undergone extensive reduction and fusion. As nearly as one can determine by a study of surface views and cross-sections, the following changes have taken place. The cephalic lobes, oesophagus, and chelicerae have disappeared, leaving hardly any recognizable traces behind. The second thoracic appendage on the left is nearly normal, that on the right, a low, irregular papilla. Between the two, in sections, traces of a double nerve-cord may be seen. Back of this point, the nerve-cords fuse and finally disappear as such, near the large, irregular that probably represents the fused appendages of the third

Following the fused appendages is a rather broad expanse, covered by a thin (single?) layer of cells.

The next thickening, *ap*⁴, is elongated transversely, and probably represents the fused appendages of the fourth segment.

The next two appendages of the left side are of full size and normal, but owing to the absence of the corresponding opposite appendages and nerve-cord they have been twisted spirally toward the right.

The abdomen is absent. At what represents the posterior end of the embryo is a deep, tubular invagination, *tp*, with thick walls, from which have arisen innumerable cells that form a dense cloud in the surrounding yolk. The embryo is therefore to be regarded as an hour-glass embryo, still further modified by the absence of the right posterior half of the thorax. It should be compared with the following one.

The surface of the embryo is covered with bacteria.

FIG. 54, $\times 60$, sectioned. This remarkable embryo in stage *D-E* belongs to the hour-glass type, but it is modified by the median fusion of the anterior end, and by the absence of the right half of the posterior end of the thorax. It seems to be the result of still further progress along the lines followed by the embryo shown in Fig. 53.

In such cases as this it is very difficult to determine just what changes have taken place, especially as regards the amount of nerve tissue, if any, that is left, and the segments to which the remaining appendages belong.

As near as can be determined, the changes have been as follows: The cephalic lobes are absent, and in their place is a broad, triangular, ectodermic plate, beneath which is an unusually large number of yolk cells.

The marginal fold has contracted anteriorly to form a thick rim around an oval depression. At the anterior end of this depression is seen in surface views a dark spot, which is the optical section of a very long oesophagus-like tube extending vertically into the yolk, about as far as the adjacent appendage is long. The tube is not closed at its inner end, and its walls are folded longitudinally, as in a true oesophagus.

Back of this tube is a long irregular appendage arising at first directly upward from the bottom of the depression, and then bending over to the side, so as to lie flat on the surface of the egg. Back of this is another appendage; it is bent double, and in such a way that its distal end points toward the median line, toward a point a little in advance of its proximal end.

The lateral growth of these two appendages at first seemed to me to indicate that the whole right half of this portion of the embryo had disappeared, and that the left half had rotated on its own longitudinal axis 180° toward the right. But after a careful examination of the sections, I failed to find any traces of either nerve-cord; and, on the whole, the sections seemed to indicate that each appendage had been formed by the fusion of a pair, and, as is so frequently the case in such embryos, the nerve-cord had entirely disappeared.

The posterior end of the embryo, which is separated from the anterior one by thin, characteristic layers of ectoderm and mesoderm, consists of a deep, thick-walled and invaginated structure. The anterior wall of this structure arises two long-pointed appendages extending outward. The middle parts of the wall of the anterior end of the embryo are composed of a single layer of cells, but the whole layer is so folded that nothing can be seen to this point could be



determined with certainty. There is, however, little doubt in my mind that the two appendages in question are the fifth and sixth thoracic appendages of the left side. The sixth has been moved in the plane of the paper 45° toward the embryo's right side. The cause of the rotation is to be sought, as in Fig. 53, in the absence of the posterior right half of the thorax.

The mesodermic area is much contracted, and in place of the continuous thickened rim are isolated masses of closely packed nuclei, some of which lie quite deeply in the yolk; others are continuous with the surface mesoderm. The masses are irregular in shape, but are usually provided with radiating streamers containing many nuclei.

FIG. 55, $\times 60$, sectioned. This is also an *hour-glass embryo*, but one in which the two parts are completely separated. The cephalic lobes are absent. The second pair (chelicerae?) of thoracic appendages have fused in the median line. The next pair are widely separated, and following them is a second pair of fused appendages. Between the unfused appendages is a well-developed double nerve-cord, which gradually narrows at either end to an unpaired cord. One end lies just back of the anterior median appendage and the other in front of the posterior one.

Back of these four appendages is an area devoid of external features. In sections it is seen to be composed of a slightly thickened homogeneous layer of ectoderm with a similar underlying layer of mesoderm. At the posterior end of this area is a deep, tubular invagination or telopore, *i.p.*, directed vertically into the yolk, and surrounded by the usual cloud of migrating cells. In front of the telopore are two small depressions, *i.a.*, that may represent invaginated appendages.

Projecting forwards over the telopore is a broad conical process. It probably represents a tail lobe such as is seen in Pl. VI, Figs. 48 and 49.

Back of this lobe is a broad convex area, composed of slightly thickened ectoderm and mesoderm, and evidently formed by the concrescence of the posterior margins of the mesodermic area. The rest of the mesodermic area is roughly A-shaped, the two backwardly directed lobes showing notches produced by partial concrescence.

FIG. 56, $\times 30$, not sectioned. This embryo is in about stage C.

The remnants of the right and left sides have undergone complete median fusion. There is now nothing left but an anterior, oesophagus-like tube, *oe.*, opening beneath a backwardly directed fold, and two median appendages. The posterior one is the larger, and projects backwards a short distance over the floor of the depression in which both lie. This depression is roughly boat-shaped, but wider and considerably deeper at the posterior end.

The nervous system is entirely absent, or, at any rate, the ectoderm over the bottom of the furrow is not thicker than, or in any way distinguishable from, that covering other parts of the embryo. The mesoderm is much thickened under what may be considered the body of the embryo, as shown by the dark rim in the figure. But a thin layer of mesoderm extends much farther than this, and along its periphery may be seen, in surface views, irregular star-shaped masses of cells, deeply imbedded in the yolk, but probably derived from the disintegrated, thickened margin of the mesodermic area.

As seen in sections, the tissues appear to be perfectly normal and healthy.

There is an indication of a transverse constriction between the two appendages. As similar constrictions occur most frequently between the third and fourth

segments, it is possible that the appendages on each side of this constriction belong to the third and fourth segments.

FIG. 57, $\times 60$, not sectioned. This is an embryo in stage *C* that has undergone partial median fusion, accompanied by transverse constriction. It could not be determined which was the anterior end of the embryo. It has been placed in its present position on account of the hood-like fold at what is now the anterior end, as it resembles a fold that sometimes covers the cephalic lobes of other embryos. At the anterior end of the embryo are two pairs of appendages nearly fused. Back of them is a thin layer of undifferentiated ectoderm with a thick mass of underlying mesoderm, the latter causing the dark color of the preparation. There is a longitudinal fold of ectoderm, probably the displaced marginal fold, on the left side of this area, and in about the middle of the same is a conical invagination, *i.e.*

At the posterior end, the fold extends around a second depression, from which arises a large protuberance, evidently formed by the fusion of a pair of appendages. There is no trace of a nervous system in this embryo, or any distinguishable lateral boundaries to the mesodermic area.

FIG. 58, $\times 60$, sectioned. Here we have a thick, oval mesodermic area, from whose inner surface several pseudopodia-like bands of nuclei extend vertically into the yolk. There are no appendages or nerve-cords, but there is an axial, ectodermic thickening, which, at what we may call the anterior end, forms a large pyramidal elevation, containing a large number of degenerating nuclei. This elevation probably represents one or more pairs of fused appendages. At the posterior end, the axial thickening gradually culminates in a tub-shaped elevation with a slit-like depression on its upper surface. At the bottom of the slit is a small circular depression. Underlying the whole is a great mass of mesoderm cells.

FIG. 59, $\times 60$, sectioned. In this embryo the cephalic lobes are hardly distinguishable. There are three small appendages on the right and four on the left side. The identification of the appendages can be approximately determined by the presence on the right of the dorsal organ. There is a great depression across the posterior thoracic and abdominal region, and below its thickened ectodermic floor, and apparently arising from it, are a great many free-yolk cells.

The mesodermic area is nearly circular. Along the whole extent of its thickened margin arises a cloud of isolated cells that extends deeply into the yolk.

The concurring posterior margins of the mesodermic areas are very beautifully shown. The median limbs, *c.m.a.*, are the most conspicuous in surface views. Sections show that this is due to the presence of a ridge-like thickening of the ectoderm, continuous with a compact band of underlying mesoderm.

On the peripheral margin of the mesodermic area the ectoderm is thin, and the underlying mesoderm is composed of isolated, lymphoid cells. The dark spot on the right, where the third appendage should be, is due in part to a small protuberance there, but in the main to the presence, *below the surface, of an oval sac with clear-cut walls.* It has the appearance of an enlarged mesoblastic somite.

FIG. 60, $\times 60$, sectioned. This is probably a very old embryo, for there are thick layers of chitin over the tips of the remaining appendages, such as is only seen in embryos ready to hatch. It at first seemed probable that the small depression, *tp.*, was the remnant of the cephalic lobes. But on sectioning the embryo, the thickenings at what is now the upper end seemed to be, without much

doubt, the brain and optic ganglia. The deviations from the normal, then, in this embryo are as follows: The whole right optic ganglion and a part of the right cerebral hemisphere are absent. On the left side the cephalic lobe is perfect, and one can distinguish in sections the minute pit that develops into the lateral eye, *l.e.* The chelicerae are probably absent, the two pairs of appendages now present belonging to the second and third thoracic segments. The appendages of the fourth segment have fused in the median line, and the marginal fold has closed in behind them. From this point in the marginal fold a faint cloud of yolk cells extends backward a considerable distance to a small thick-walled depression in the ectoderm, *tp.* From the posterior wall of the shallow depression arises a minute papilla, which may be the remnant of the tail lobe. (Compare Figs. 27, 43, and 64.) It seems too far back to represent the fused appendages of the last thoracic segment. The anterior part of the depression is still further invaginated to form a short, conical pocket, directed diagonally forward into the yolk. The inner end of the pocket is continuous with a great mass of cells which seem to be wandering into the yolk there to degenerate and disappear. It would appear, therefore, that transverse fission has taken place, and that contrary to every other case that has come under my notice, except, perhaps, Fig. 45, the subsequent fusion and degeneration have been greatest in the posterior half of the thorax.

FIG. 61, $\times 60$, sectioned. The mesoderm and ectoderm are much thickened and concentrated along the axial portion of the embryo. There is no distinction into head, thorax, and abdomen. The body of the embryo forms a deep, elongated furrow, constricted laterally to form a chain of four or five clearly marked dilatations, each one of which represents a metamere. At the bottom of two of the dilatations are shallow, slit-like infoldings that represent the remnants of invaginated thoracic appendages, *ia.* In sections there is no recognizable remnant of the nerve-cords to be seen.

The mesodermic area is sharply defined on its periphery, where there is the usual thickened rim, but it contains very few mesoderm cells, except beneath the axial portion, where they form a very thick, compact layer ten to fifteen cells deep. It is this deposit of cells which forms the dark ring seen in surface views.

This embryo is also remarkable for the fact that the surface ectoderm of the entire mesodermic area is covered with a very thick deposit of a soft, vitreous exudation, resembling chitin. Over the axial furrow it has accumulated in great botroidal masses, among which in each section may be seen four or five rounded, amoeboid cells that look like those seen in the peripheral vesicles. There is no indication as to where these isolated cells, which are separated by considerable distance from the embryo, come from, or what their function may be. Their occurrence at this place suggests a rudimentary amnion and serosa, but otherwise there is nothing to indicate that they develop in the manner characteristic of these organs.

FIG. 62, $\times 60$, not sectioned. Embryo with three pairs of appendages, perhaps the fourth to sixth pairs. The cephalic lobes are deeply depressed, and separated from the thorax by a sharp, transverse fold. The rim of the mesodermic area is very distinct, and shows clearly the posterior conrescing margins, and the post-anal cloud of yolk cells between. *p.a.c.*

The abdomen is deeply depressed, forming a cavity with nearly vertical walls surrounding a triangular opening.

FIG. 63, $\times 50$, not sectioned. This embryo is shown as an opaque object by reflected light. It was drawn shortly after it was killed in picronitric acid, and while still in the fluid.

The embryo is small, but has six pairs of thoracic appendages and well-developed cephalic lobes. It shows in a very striking manner a conspicuous ridge extending completely around the embryo. It is broadened and thickened posteriorly, and is continuous, with a thick, broad elevation extending forwards to the posterior portion of the body. This ridge is the very well-developed marginal thickening of the mesodermic area, such as has been shown in many of the preceding figures. The post-anal band is formed by the concrescence of the posterior margin of the mesodermic area. When seen in this way, as an opaque object, its resemblance to the blastodisc of a vertebrate embryo is obvious (shark, lizard, or chick).

FIG. 64, $\times 60$, not sectioned. A peculiar embryo without appendages, but showing three pairs of distinct, hollow, mesoblastic somites. In shape it resembles the embryos of scorpions or spiders more than those of *Limulus*.

FIG. 65, $\times 60$, sectioned. A small, abbreviated embryo in stage C. The rudimentary cephalic lobes are depressed and rotated to the embryo's right. Their lateral margins are still further invaginated to form two oval depressions, nearly concealed by distinct folds that have grown over them from the sides, *b. & v.* The oesophagus is comparatively large, and the slit-like opening is also rotated toward the left.

The chelicerae are probably absent. On the left are the fourth (?) and sixth (?) appendages, but the fifth is invaginated. On the right the fourth (?) and fifth (?) are invaginated, and the sixth (?) persists.

A telopore is present behind the last pair of appendages. Back of the embryo is seen a long, faint band of yolk cells, *p.a.c.*, formed by the concrescence of the margin of the mesodermic area.

FIG. 66, $\times 60$, not sectioned. A small embryo in stage C.

The cephalic lobes are not distinguishable as such, but the oesophagus is distinct. There are three appendages (fourth, fifth, sixth[?]) on the left, and two on the right. In place of the posterior part of the thorax is a broad depression.

FIG. 67, $\times 60$, not sectioned. A narrow embryo, depressed along the median line, and surrounded by high and sharp marginal folds. The cephalic lobes are depressed below the level of the thoracic nerve-cord. The second (?) and third (?) pairs of thoracic appendages are present with one more appendage on the right. In the abdominal region is a deep, pyramidal depression, directed nearly vertically downwards.

The dark area along the marginal fold of the embryo is produced by unusually thick layers of mesoderm. The margin of the mesodermic area is not clearly defined; it lies, in part, at least, beyond the limits of the figure. On the left side of the embryo, in the thickened mesodermic margin, is a large, marginal vesicle. The two dark areas back of the abdomen are formed by longitudinal, ridge-like thickenings of the ectoderm and mesoderm. Between the two ridges is a depressed area covered by a thin layer of mesoderm and ectoderm. It appears, therefore, that the posterior median margins of the mesodermic area have not completely united, except at their very posterior ends. In front of this point is a rather large rhomboidal space not covered by the mesodermic area.

FIG. 47, $\times 60$, not sectioned. The first and second thoracic segments are absent. The cephalic lobes, however, persist as a faint disc of cells without character. This case differs somewhat from the preceding ones, in that the effects of degeneration do not increase gradually from before backward, for degeneration has been greater in the first two post-oral segments than in the cephalic lobes.

The median space between the appendages of the remaining pairs gradually increases toward the posterior end.

A still further difference between this embryo and the preceding ones is seen in the fusion across the median line of the abdominal appendages, the last appendage being the smaller one.

FIG. 48, $\times 60$, sectioned. In this embryo, the sixth pair of thoracic appendages are identified by the well-developed flabella. The long median appendage is formed by the fusion of the fourth pair of the thoracic appendages, the unfused tips of the two original appendages being still visible. The first three pairs of appendages and the entire cephalic lobes have disappeared. The nerve-cord is normal and well developed from the root of the tail lobe to the base of the unpaired appendage of the fourth segment. The abdomen is small, but possesses two pairs of normal appendages. There is a very conspicuous tail lobe, the size and shape of which suggest the idea that with the diminution in size of the embryo, the marginal fold became too large, and the excess accumulated at the posterior end to form the tail lobe. *Back of the tail lobes is a small conical projection, also visible in the sections, having all the appearance of an unpaired thoracic appendage.* The presence of an appendage in this place is very remarkable, and I am unable to offer any explanation for its occurrence there.

The body of the embryo forms the floor of a deep depression bounded by the marginal fold. The embryo stained very deeply, as it is composed of dense tissue containing a large amount of chromatin. Anteriorly, the rim of the mesodermic area, which was not visible, or perhaps overlooked in the surface views, is easily seen in the sections, apparently preserving its normal size and extension for this stage. In the sections that pass a long distance in front of the present anterior end of the embryo, the mesodermic rims are seen as widely separated as in the normal embryo of this stage. In the line midway between them, instead of the nerve-cords and appendages, there is merely a thin, undifferentiated layer of ectoderm with a few scattering mesoderm cells beneath it.

FIG. 49, $\times 60$, sectioned. The cephalic lobes and first two thoracic segments are absent. No trace whatever of these organs is to be seen in sections, although there is a dark area, due to an accumulation of loose cells, where the anterior end of the embryo should be.

The marginal folds, *m.f.*, are distinct and well developed, and extend across the median line just in front of the second pair of thoracic appendages. The latter are fused with each other at their bases, and just back of them the nerve-cord terminates abruptly in a blunt, unpaired process.

The abdomen is very well developed, and terminates in a broad tail lobe that projects upwards and forwards, thus overarchng the posterior part of the abdomen.

Such a prominent tail lobe, although occasionally seen, is not a common form of abnormality. It is suggestive of the prominent tail fold in insects and crustacea, but in this peculiar case recalls that seen in scorpion embryos.

59.



45.

morch.



mat.

ap³



48.

mt

crd

49.

x



mt



FIG. 47, $\times 60$, not sectioned. The first and second thoracic segments are absent. The cephalic lobes, however, persist as a faint disc of cells without character. This case differs somewhat from the preceding ones, in that the effects of degeneration do not increase gradually from before backward, for degeneration has been greater in the first two post-oral segments than in the cephalic lobes.

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Such a prominent tail lobe, although occasionally seen, is not a common form of abnormality. It is suggestive of the prominent tail fold in insects and crustacea, but in this peculiar case recalls that seen in scorpion embryos.

It is very interesting to note that these two cell masses resemble in position and general appearance the two proliferating areas, "primitive cumuli," which at a very early period give rise to the head and trunk of normal embryos.

No trace of primitive cumuli is visible in normal embryos after stage *C*, and as these embryos are certainly as far advanced as that stage, they either must have retained to this late period a very early embryonic character, or else the processes of degeneration have carried them back a second time to their original condition. In either case, the facts seem to emphasize the morphological importance of these two proliferating centres.

There is no very reliable indication as to what is the head or tail *Anlage*, except perhaps in Fig. 73. The embryos have been oriented in each case with the smaller *Anlage* at the anterior end, and arranged and numbered as far as possible in accordance with the degree of degeneration.

It is obvious that such a plan of arrangement cannot be followed with any accuracy, since each embryo has its own way of degenerating, begins to degenerate at different stages of development, and may have been defective at the outset. What finally happens to these degenerated embryos cannot be determined, since further degeneration of either head or tail *Anlage* would make it impossible to determine whether it belonged to this series or the next. But I see no reason to doubt that degeneration goes on till even the head and tail *Anlagen* are absorbed and disappear completely.

While Fig. 73 is the first one of the series here represented, numerous intermediate stages between it and normal embryos of stages *C* and *D* were found.

Just how far normal development may proceed before this general degeneration begins could not be determined. I do not recollect seeing any embryo undergoing this phase of degeneration, which in whole or in any part had reached a higher stage of development than that characteristic of stage *D*. But it must be borne in mind that all these embryos had been developing or degenerating in the same jars with normal eggs, for from fifteen to eighteen weeks. The normal ones passed through their whole development and escaped from the jars as trilobite larvae, in but little more than half this time.

FIG. 72, $\times 60$, sectioned. In this embryo, every trace of cephalic lobes, oesophagus, nerve-cords and appendages has disappeared. The mesodermic area is very large and irregular. Anteriorly the margin of the same is pretty close to the axis of the embryo. It there consists of numerous star-shaped masses of cells, densely crowded together, *m.a.* The posterior margins are much thickened to form two great diverging arms, which are united at their distal extremities, but are separated medianly by a triangular area devoid of mesoderm cells (compare Fig. 67). These thickened arms unquestionably represent the posterior margins of the mesodermic area which has not completely concresced along the median line. It is important to recognize this, because it shows approximately to what a late stage of development this embryo belongs. Such embryos as this and those that follow on this plate, cannot for a moment be considered as belated normal embryos, in early stages of development. Their great age (two or three months), the concrescence of the mesodermic margins, the histological character of the tissues, and a comparison with the early stages of normal embryos, is sufficient to prove this beyond any question.

A thin layer of ectoderm covers the greater part of the mesodermic area, but it is thickened and elevated in the axial region, over what probably represents the

body of the embryo. The elevation forms a broad, figure-8-shaped ridge (marginal folds?), with a slit-like depression in about the centre of each loop. There is a thickened layer of mesoderm beneath the whole of this axial portion.

Beneath the anterior end is a very large solid mass of mesoderm cells, *c.m.s.*, from which pseudopodia-like streamers of cells radiate deeply into the yolk.

FIG. 73, $\times 60$, sectioned. In this embryo the tissues are beautifully clear, and sharply differentiated, resembling histologically the late stages in the formation of the blastoderm of normal embryos. The ectoderm, instead of containing flattened cells with little protoplasm, is composed of high columnar cells with nuclei at their outer ends, their inner ends being filled with masses of yolk granules. The whole layer is sharply separated from the underlying yolk and mesoderm.

There is no cluster of yolk cells at the anterior end. The dark median band consists of columnar ectoderm cells, like those described above, but higher, and below them is a clear band of mesoderm. Both layers are entirely undifferentiated.

At what seems to be the posterior end, is a deep, oblong depression with two infoldings of its anterior wall, like steps leading down into it. Numerous degenerating nuclei arise from the inner surface of the infolded layer, and lie scattered about in the neighboring yolk. The mesodermic area is circular, with a faint thickening of its anterior and lateral margins. There is no trace of a posterior concrescence.

FIG. 74, $\times 60$, sectioned. Embryo similar to the preceding. It consists of two disc-shaped ectodermic thickenings, connected by a longitudinal band of the same nature. Beneath the thickened ectoderm are corresponding mesodermic thickenings, but especially enlarged at either end.

There is a shallow depression in the centre of each ectodermic disc. In both Figs. 74 and 76 the ectoderm of the posterior thickening shows a higher grade of histological differentiation than those of the anterior one.

The peripheral outline of the mesodermic area was not visible.

FIG. 75, $\times 60$, sectioned. An embryo with a circular, mesodermic area, and a "blastopore"-like invagination at either end.

The invaginations are similar to those already described, except that comparatively few yolk cells surround the invaginations. The outer surface of the invagination is covered with a thick layer of bacteria (?). The ectoderm is a uniformly thin layer of cells, showing no trace whatever of a median thickening connecting the two invaginations. The mesoderm is remarkable in that it is of nearly uniform thickness throughout its whole extent, except at the margin where it is decidedly thickened, *m.a.*

Most of the marginal mesoderm is composed of the characteristic, striated cells seen in stage D, and later.

FIG. 76, $\times 60$, sectioned. This embryo consists of a circular, mesodermic area containing scattered, star-shaped masses of cells, either extending from the mesoderm into the underlying yolk, or lying freely in it. There is a deep, circular, ectodermic invagination at the anterior end with a thick layer of mesoderm surrounding it, and a similar, but larger, deeper, and more irregular cavity, at the posterior end. The ectoderm lining this invagination is considerably thicker than that of the anterior one, and the thickening extends along the surface for some distance back of the infolding, *p.a.c.* This thickening is probably the result of the concrescence of the posterior margins of the mesodermic area. There are

EXPLANATION OF PLATE VII.

FIG. 50, $\times 60$. This embryo evidently represents an extreme case of median fusion, but differs from those on the preceding plate in that there are no indications of that V-shaped arrangement of paired organs usually seen when a progressive antero-posterior degeneration has followed the median fusion. The cephalic lobes, oesophagus, and nervous system are entirely absent. Nothing remains of the body but an oblong elevation of thickened ectoderm with three median appendages arranged along its summit. Behind the third median appendage was a partly invaginated plug of cells, *t.p.*, that may be the remnant of the telopore. Besides this there was nothing to indicate to what segment these fused appendages belonged, or which was the anterior and which the posterior end of the body. The mesodermic area was slightly raised and was pentagonal in outline, with a thickened rim.

FIG. 51, $\times 60$. A large embryo in stage *C* showing a distinct transverse constriction between the third and fourth thoracic segments.

The anterior part of the embryo is normal, except in the absence of the chelicerae.

The posterior portion is infolded between the appendages of the sixth thoracic segment to form a deep, circular cavity. The sixth pair of legs, identified by the flabella on their outer margin, have been carried inwards by the infolding, till they project toward each other from its lateral walls.

FIG. 52, $\times 60$, sectioned. An hour-glass embryo in stage *C*.

The cephalic lobes are deeply depressed, and nearly covered by two lateral folds. The chelicerae are absent, the next two appendages are brought closely together, and the third pair completely fused to form a thick median appendage, *ap*³, extending backwards.

These three appendages project from a circular depression, bounded on all sides by a thickened margin, which anteriorly forms the folds overlapping the cephalic lobes.

The fourth pair of appendages are also fused, *ap*⁴, and the fifth are either absent, or fused and invaginated to form the pit just back of the base of the preceding pair, *ap*⁵.

The sixth pair are small and devoid of flabella, but are nearly normal in shape and position.

The posterior part of the thorax and a part of the abdomen are nearly surrounded by a marginal fold, well developed posteriorly, but not extending across the median line in front of the fused appendages of the fourth segment.

FIG. 53, $\times 60$, sectioned. A remarkable embryo in stage *C* that has undergone extensive reduction and fusion. As nearly as one can determine by a study of surface views and cross-sections, the following changes have taken place. The cephalic lobes, oesophagus, and chelicerae have disappeared, leaving hardly any recognizable traces behind. The second thoracic appendage on the left is nearly normal, that on the right, a low, irregular papilla. Between the two, in sections, traces of a double nerve-cord may be seen. Back of this point, the nerve-cords fuse and finally disappear as such, near the large, irregular, median protuberance that probably represents the fused appendages of the third segment, *ap*³.

Following the fused appendages is a rather broad expanse, covered by a thin (single?) layer of cells.

The next thickening, ap^4 , is elongated transversely, and probably represents the fused appendages of the fourth segment.

The next two appendages of the left side are of full size and normal, but owing to the absence of the corresponding opposite appendages and nerve-cord they have been twisted spirally toward the right.

The abdomen is absent. At what represents the posterior end of the embryo is a deep, tubular invagination, tp , with thick walls, from which have arisen innumerable cells that form a dense cloud in the surrounding yolk. The embryo is therefore to be regarded as an hour-glass embryo, still further modified by the absence of the right posterior half of the thorax. It should be compared with the following one.

The surface of the embryo is covered with bacteria.

FIG. 54, $\times 60$, sectioned. This remarkable embryo in stage *D-E* belongs to the hour-glass type, but it is modified by the median fusion of the anterior end, and by the absence of the right half of the posterior end of the thorax. It seems to be the result of still further progress along the lines followed by the embryo shown in Fig. 53.

In such cases as this it is very difficult to determine just what changes have taken place, especially as regards the amount of nerve tissue, if any, that is left, and the segments to which the remaining appendages belong.

As near as can be determined, the changes have been as follows: The cephalic lobes are absent, and in their place is a broad, triangular, ectodermic plate, beneath which is an unusually large number of yolk cells.

The marginal fold has contracted anteriorly to form a thick rim around an oval depression. At the anterior end of this depression is seen in surface views a dark spot, which is the optical section of a very long oesophagus-like tube extending vertically into the yolk, about as far as the adjacent appendage is long. The tube is not closed at its inner end, and its walls are folded longitudinally, as in a true oesophagus.

Back of this tube is a long irregular appendage arising at first directly upward from the bottom of the depression, and then bending over to the side, so as to lie flat on the surface of the egg. Back of this is another appendage; it is bent double, and in such a way that its distal end points toward the median line, toward a point a little in advance of its proximal end.

The lateral growth of these two appendages at first seemed to me to indicate that the whole right half of this portion of the embryo had disappeared, and that the left half had rotated on its own longitudinal axis 180° toward the right. But after a careful examination of the sections, I failed to find any traces of either nerve-cord; and, on the whole, the sections seemed to indicate that each appendage had been formed by the fusion of a pair, and, as is so frequently the case in such embryos, the nerve-cord had entirely disappeared.

The posterior end of the embryo, which is separated from the anterior one by thin, characterless layers of ectoderm and mesoderm, consists of a deep, thick-walled and irregular sac, from the posterior wall of which arise two long-pointed appendages, extending upward and forward. There are some parts of the wall of the sac that look histologically like parts of a nerve-cord, but the whole layer is so folded and crowded together that nothing in regard to this point could be

has apparently persisted as a large, conical lobe, projecting almost vertically upwards from the surface of the yolk. It is evidently the same kind of a lobe seen in Figs. 13, 48, and 49. The body of the embryo, as in the three preceding figures, is reduced to a mere sac. The opening of the sac is triangular, and almost as wide as the sac is broad. A little to one side, on the floor at the anterior end, is a small, tubular invagination, oesophagus (?), directed vertically downwards.

FIG. 83, $\times 60$, sectioned. This is another important embryo. The mesodermic area is relatively small, and not very sharply defined anteriorly, owing to the small number of mesoderm cells contained in it. Posteriorly, and this is a point of value in determining the poles of the embryo, the mesodermic margin is decidedly thickened, and shows very clearly the effects of concrescence, *p.a.c.* The axial ectoderm shows the usual thickening, and is invaginated to form a complicated, thick-walled sac, opening by an oval aperture. The sac projects forwards beneath the ectoderm, and at its anterior end expands into two transverse tubes. If the nerve-cord and the cephalic lobes are still present, although they cannot be recognized as such, it is obvious that the floor and sides of the sac will consist of these organs, while the lateral eyes would be situated somewhere near the end of the lateral diverticula. This condition is what normally obtains in vertebrates, and is of obvious significance in view of the other resemblances which we have pointed out elsewhere, between the normal brain and eyes of *Limulus* and those of vertebrates. Such embryos are extremely rare, and may have no morphological value. But the possible significance of such variations should not be overlooked.

Beneath the aperture of the sac, the floor of the same suddenly slopes downward, and at the bottom of this depression is still another smaller and tubular one that projects downward and forward into the yolk. The character of the tissues, which seem to be perfectly normal, and further structural details, are shown in the three transverse sections (Pl. X, Figs. 83^{1, 2, 3}).

FIG. 84, $\times 60$, sectioned. Of the mesodermic area, which is nearly circular, only the posterior portion is represented. The whole area consists of a thick layer of rounded, and often isolated, lymphoid cells, lying in a space between the yolk and a thin layer of ectoderm. There is a prominent marginal thickening extending completely around the mesodermic area. In the centre of the latter the ectoderm is thickened and deeply invaginated to form a roughly cubical cavity, partly closed over in front by a backwardly directed lip, or fold, and behind by a similar one directed forwards.

The thick walls of this cavity probably represent that part of the embryo from which the nerve-cords and appendages are developed, the whole complex mass being reduced to a continuous, undifferentiated layer.

From the anterior wall of the cavity projects a short, tubular outgrowth that looks very much like an embryonic stomodaeum, the resemblance being increased by the presence of a *pair of muscle strands*, which extend laterally from the tip of the tube to the surface ectoderm. The presence of this specialized tissue offers a sharp contrast to the very early embryonic character of the rest of the embryo.

This fact supports my view, if further evidence were necessary, that these are very old embryos whose organs have been reduced, with this exception, to the simplest embryonic tissues.

FIG. 85, $\times 60$, sectioned. The mesodermic area here is small, circular, and poorly defined. The margin, however, at the anterior border is greatly enlarged

to form a marginal vesicle, *m.v.* In the centre is a kite-shaped sac opening to the surface by an elongated figure-8-shaped opening, *c*. The anterior part of the floor of the sac protrudes upwards and forwards (cephalic lobes[?]), and from this inclined wall projects forwards a short tube or stomodaeum(?), *b*. Both structures are seen in sections in Pl. X, Fig. 85^{1,2}. The sac extends backwards some distance beyond the aperture, *c*, as a cylindrical tube which ends blindly deep in the yolk.

There is a shield-shaped thickening of the ectoderm, lying over and a little to the right of the sac. It is represented by the shaded area, *d*. A section through this thickening and the posterior end of the sac is shown in Fig. 85².

FIG. 86, $\times 60$. This embryo is similar to the preceding one. Unfortunately, it was lost or injured before it could be sectioned. In the centre of the mesodermic area is a thick-walled sac, which communicates with the exterior by a diamond-shaped opening. The anterior end of the sac is very broad and spacious, and the posterior part a deep, nearly closed furrow terminating in a tube that dips vertically into the yolk. The relation of these parts, so far as they could be determined in surface views, is shown in the accompanying median, longitudinal, optical section. Fig. 86, *B*.

FIG. 87, $\times 60$, not sectioned. The mesodermic area appears to be nearly circular, but its outline is very indistinct. In its centre is a conical, thick-walled projection, with an opening at the summit leading into a large, nearly spherical sac. Specimens resembling this one in all but unimportant details are comparatively common. They are evidently further modifications of the conditions seen in the five preceding figures. This specimen was not sectioned, as it did not appear to present any noteworthy features.

FIG. 88, $\times 60$, sectioned. In this case a sac like that seen in the preceding figures is beginning to break up. The mesodermic area is distorted, and on the left ill defined; on the right the rim of the mesodermic area shows the characteristic, star-shaped masses of cells that are spreading out into the yolk, preparatory to their final dissolution.

In sections of other specimens, apparently in a similar condition to this one, the walls of the sac have lost their sharp outlines, as though they were gradually falling apart; and the cells composing them have the character of closely packed, lymphoid cells, rather than that of the columnar ones seen in the preceding figures. Still one would hardly suspect, from a mere inspection of the sections, that these specimens were anything else than very early stages of normal embryos.

FIG. 89, $\times 60$, sectioned. In this remarkable specimen we have a good example of the last stages in the degeneration of the embryo. In all the other cases I have studied, where the thick walls of the sac had broken up into a formless mass of cells, the latter were usually rounded, lymphoid ones, showing no further differentiation. But in this instance the entire mass, representing all that is left of the embryo, is composed of those peculiar cells, containing coiled filaments that are only found at a late embryonic period in the thickened margin of the embryonic area. I have already referred to these cells in one of my earlier papers ("Origin of Vertebrates from Arachnids," p. 375), and at that time supposed they were purely embryonic, and gave rise in some instances to the future muscle cells. Since then, however, I have found them as free, amoeboid cells, in great numbers in the tissues of the adult animals. They differ somewhat in histological characters from the embryonic ones of the mesodermic margin, but there can be no question whatever about their identity. In the adult these cells are very

abundant all through the tissues about the median and lateral eyes and the olfactory organs, and I see no reason to doubt that they will be found as abundantly elsewhere. Kingsley failed to confirm the existence of these cells in the embryo, which is somewhat surprising, for in stage *D* they form as conspicuous an object in sections and surface views as any other organ of the body.

I expect to describe in more detail the history of these cells in another paper. I refer to them here to make clear the remarkable fact that in these degenerate embryos there is almost nothing left but a mass of these highly specialized and peculiar cells. The only thing remaining besides them are the ordinary yolk cells and the blastoderm, and even this disappears, as such, over the mass of cells under consideration. It is as though a mammalian embryo should gradually degenerate into a mass of cells consisting solely of osteoblasts, or muscle cells, or any other specialized form of tissue. However, it must not be assumed that other kinds of cells are during degeneration converted into the fibre cells. The latter merely persist as such after the others have disappeared.

On careful study of one of the sections we see the fibre cells usually in ill-defined groups, with intermediate areas containing some nuclei, unquestionably in karyokinesis. Other nuclei, however, seem to have made preparations for division, but instead of doing so they break into numerous deeply stained globules, which become disseminated through the yolk, and growing fainter and fainter, finally disappear.

A portion of a section through the embryo is shown in Pl. X, Fig. 89. Below the disc is a clear area containing a few yolk globules, and many degenerating nuclei and cells, apparently derived from the mass of cells above.

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1907



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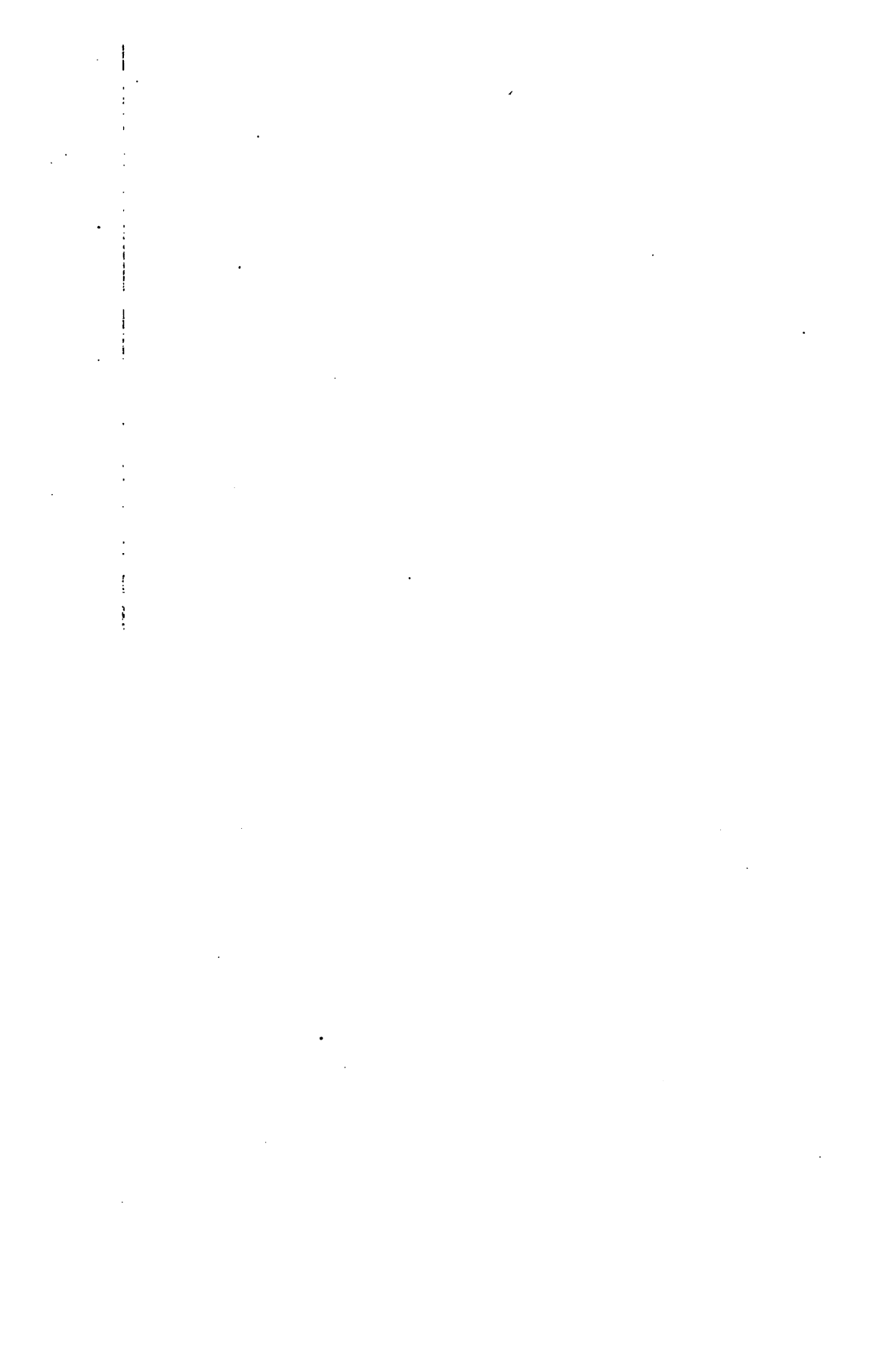


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EXPLANATION OF PLATE IX.

In this plate, double monsters are shown in various stages of formation. In all the cases I have seen in *Limulus*, double embryos are formed by fission, or more correctly by the gradual intercallation, beginning at the anterior end, of two new halves between the old. If there are five paired organs on each segment, and *a* is the most median one, and *c* the most lateral, then *a* will be the first new organ to appear, and it will appear in the median line of the first segment, as an unpaired organ, having the same appearance as each member of the paired organ. It divides, and in its place in the same segment will be found an unpaired organ like organ *b*. But at the same time a new, unpaired organ, like *a*, will be formed in the median line of segment number two. At the next division, organ *a* will be produced in the median line of the third segment, *b* in the second, and *c* in the first; organs *a* and *b* being now completely formed in pairs in the first segment, and organs *b* in the second. This process goes on till two complete new halves are wedged in between the old, and two new individuals are produced, each individual consisting of an old and a new half. The old halves were produced by normal apical growth from behind forward; the new half was also produced by apical growth, but from before backwards. Each new half is a mirror image of the other, and at the same time the mirror image of the old half next to it; but the new halves are united by their lateral margins instead of the median ones.

The very first steps in this process have not been seen. Hence the manner in which the new halves of the cephalic lobes and the oesophagus are produced, can only be inferred from the manner in which new organs are formed in the post-oral segments. The process of forming two new organs by the division of the unpaired ones, takes place in exactly the reverse order that unpaired organs are formed by the fusion of paired ones.

FIG. 90, $\times 33$. The entering wedge formed by the newly produced organs has reached the fifth thoracic segment. At the end of the wedge is an unpaired neuromere; in the next in front of it are two new halves of a neuromere and an unpaired appendage. In the next segment in front of that are two complete and newly formed chelicerae.

FIG. 91, $\times 33$. Here the process has progressed still further. The division of the new appendage in the third segment is almost completed, the separation extending from the tip nearly to the base. In the fourth segment the new appendage is perfect, but shows no trace of division.

FIG. 92, $\times 30$. The growth of the new halves is practically completed, forming two distinct embryos, which have, however, an abdomen in common. The new halves lie on the upper side, as the egg is placed in the figure, the old ones being below. Each of the old halves has been rotated on its tail end 90° , one to the right, the other to the left. They have been forced apart in this way, at first by the wedge-like ingrowth of the new nerve-cords. But as the organs lateral to the two nerve-cords develop, they push the previously formed parts still farther right and left. This goes on till the new embryos form a straight line tail to tail. Further rotation of the old halves will be stopped by the interference of the lower margins of the mesodermic areas at *y*.

EXPLANATION OF PLATE X.

FIG. 100, $\times 25$. This is one of the oldest double monsters seen. It should be compared with Figs. 96 and 98. The development of the new halves had apparently not separated the old ones much more than in Fig. 98. The main embryo has the abdomen of the original embryo.

In the smaller embryo are two unpaired, tongue-like, abdominal appendages. The appendages of the sixth segment are fused at their base, and in front of them is a row of three long, crumpled filaments, representing the medianly fused appendages of the third, fourth, and fifth segments. All the segments in front of the third have disappeared.

FIG. 101, $\times 30$. Same embryo as in the preceding figure, seen from above. The dorsal surface of the thorax of the smaller embryo is spread out on that of the larger. Below the dark mass of cells that represent the remnants of the thickened margin of the mesodermic area is an elongated cloud of cells, probably the remnant of the oesophagus, *œ*.

There is a well-developed heart in the abdominal region of the larger embryo, but none in that of the smaller one.

FIG. 102, $\times 30$. There are three embryos on this egg. Embryo *A* is normal and perfect in everything except the abdomen. *B* has undergone median fusion and degeneration, and transverse fission. The cephalic lobes and first four segments have disappeared, except two incompletely fused appendages. The abdomen and the posterior part of the thorax persists. The latter is bounded in front of the fifth pair of appendages by a great fold that extends completely across the median line. The nerve-cord in this posterior remnant of an embryo forms a conspicuous, unpaired ridge.

Embryo *C* has undergone such fusion and antero-posterior degeneration that nothing remains but the fused appendages of the sixth segment, and a rudimentary abdomen.

It is probable that the original embryo divided lengthwise, giving rise to *A* and *BC*, and the latter then divided, giving rise to *B* and *C*.

FIG. 103, $\times 30$. In this triple embryo the individuals, *A*, *B*, and *C*, were probably produced in the same way as in the preceding. As all the embryos could not be seen at once, each embryo was drawn separately with the aid of a camera, and finally all three united and represented as though spread out on a flat surface.

Embryo *A* has undergone median fusion and transverse fission. The fused appendages of the first four segments are arranged in a single row; it is very rarely that one sees as many unpaired appendages as this.

The cephalic lobes are narrowed, and covered by a hood-like fold of ectoderm, through which one sees the oesophagus.

The marginal fold has grown across the median line in front of the fourth pair of appendages, as in the extreme forms of hour-glass embryos. In front of this fold, and near the median line, are the dorsal organs.

The fifth pair of appendages have not fused entirely. Embryo *B* has degenerated completely in front of the fused fifth pair of appendages, with the exception of the dorsal organs, which have almost reached the median line.

The sixth pair of appendages have nearly fused at the base, but are distinct at the tips.

FIG. 93, $\times 33$. The process seen in the preceding figure has evidently begun here. But before the two new halves were completely formed, the left-hand embryo began to conresce along its own median line. The cephalic lobes and first two segments have disappeared in that way, exactly as in single embryos (see Pl. IV). The sixth pair of appendages has not been formed in the new halves.

FIG. 94, $\times 33$. Here the process seen in the preceding figure has been carried further. Fission probably occurred at first, as in Fig. 92; degeneration of one embryo then followed, as in Fig. 93; and finally the two embryos separated, moving tail first in opposite directions to the position they now occupy. The right-hand embryo is apparently normal; the left-hand one has been reduced by antero-posterior fusion and degeneration, till the last two pairs only of thoracic appendages remain. The fourth pair have united in the median line. The "dorsal organs," *d.o.*, are still separate and very distinct. Between them is a small mass of cells, the remnant probably of an unpaired appendage. This embryo, with its five legs and large tail lobe, is very similar to that in Pl. V, Fig. 48.

FIG. 95, $\times 33$. This is a double embryo which, at one stage of its development, was probably like that in Fig. 92. The normal upper embryo still occupies its former position tail to tail with the lower one, which median conrescence and antero-posterior degeneration has reduced to a small, median appendage, projecting from an oval depression. The abdomen is a narrow oblong thickening, and at the opposite extremity of the body is a median depression that may represent the fused dorsal organs (compare Fig. 97), or the remnants of an oesophagus.

On either side of this much-reduced embryo is an irregular, dark band, formed by the conrescence of the margins of the mesodermic areas of the two embryos, and probably representing two rudimentary hearts.

FIG. 96, $\times 33$. This is a much older embryo than any of the preceding. It has passed successively through approximately the same stages seen in Figs. 92-94. The exact sequence of events, of course, cannot be determined; but we can understand the position of appendages in the right-hand embryo, by assuming that after a tail-to-tail condition was reached median fusion and antero-posterior degeneration of the lower embryo followed, somewhat as in Fig. 93. Then the tail of the lower embryo pushed past the tail of the upper, going to the right of it instead of to the left, as in Fig. 94. But meantime the margin of the thorax of the larger embryo had grown so large that it had practically preëmpted the territory in that region; the tail of the smaller embryo was thus forced to bend to the right, through an angle of about 90° . The curvature thus produced of the axis of the smaller embryo is shown by the arrow *x*, and by the dotted line which runs along the median line from the fourth thoracic appendage, *ap*⁴, to the abdomen. The latter is perfectly normal and well developed. The cephalic lobes and first three neuromeres have disappeared, through median fusion and antero-posterior degeneration. The appendages of the fourth and fifth segments have fused along the curved median line, and are very much reduced in size. The sixth pair, *ap*⁶, are still quite large, and fused only at their base. The flabella, *f.*, are very large, and each is separated by a relatively wide space from the base of its respective appendage.

Just under the end of the left sixth appendage is a small projection that cannot be accounted for, as its position is such that it cannot be brought into line with any of the other appendages. It may be the left flabellum of embryo *A*.

FIG. 97, $\times 33$. This embryo is in an older stage than the preceding one. It is an almost perfectly symmetrical double embryo, tail to tail as in Fig. 92. Median fusion and antero-posterior degeneration have affected both embryos, but the lower one more than the upper. In the latter, everything as far as the fourth segment has disappeared. The dorsal organs are very large, and are supplied on one margin with considerable black pigment, a condition that has not been seen in the normal embryos. They have approached the median line, but have not fused with each other. The appendages of that segment, *i.e.* fourth, have fused, and the single appendage thus produced is reduced to a small conical projection, *ap*⁴. The fifth pair have also fused, forming a long zigzag appendage. The sixth pair have fused at the base to form a large oval vesicle, from the summit of which project the separate ends of the reduced appendages. The chelaria and abdominal appendages are normal.

In the lower embryo, *B*, the "dorsal organs" on the fourth segment have fused. Everything anterior to that has disappeared, but the appendages of the fifth and sixth segments remain as elongated, crumpled, unpaired organs.

The nerve-cords extend without interruption or modification from one animal to the other. There are two perfectly normal hearts and two abdominal lobes, both organs being shared in common by the two embryos.

FIG. 98, $\times 33$. This is a very interesting case, as it shows clearly three entirely separate phenomena, *i.e.* (1) longitudinal division; (2) median concrescence; (3) transverse fission. The two latter conditions have been seen in the single embryos previously described. The two embryos now form almost a straight line. The abdomen of the original embryo is intact, and would have become, in all probability, the abdomen of embryo *A*. A remarkable fact is the obvious "weakness" of the new half of embryo *A*, as compared with its old, right side. This is shown by the absence of its second and fourth appendages, and the fourth neuromere. In place of the fourth appendage is a minute pore that may be the invaginated remnant of the same. Embryo *B* is a beautiful example of the hour-glass type, the constriction occurring in the usual place, between the third and fourth segments. The appendages of the third and fourth pairs are fused in the median line. In front of the third pair, and back of the fourth, is a gradual diminution of the concrescence. The large size of the chelicerae in embryo *B* is surprising, considering the otherwise reduced condition of the embryo.

FIG. 99, $\times 35$. This is a very rare form, and the only one of the kind I have seen. It at first sight seems to belong to a different class from the preceding, and to have been produced in a different manner. However, it is easily explained by assuming that during fission, like that in Fig. 91, median fusion and antero-posterior degeneration destroyed the anterior part of embryo *B* as fast as it was formed. Two new nerve-cords extended to the tip of the abdomen, and a row of unpaired appendages, extending from the fourth abdominal to the fifth thoracic segment, have been formed.

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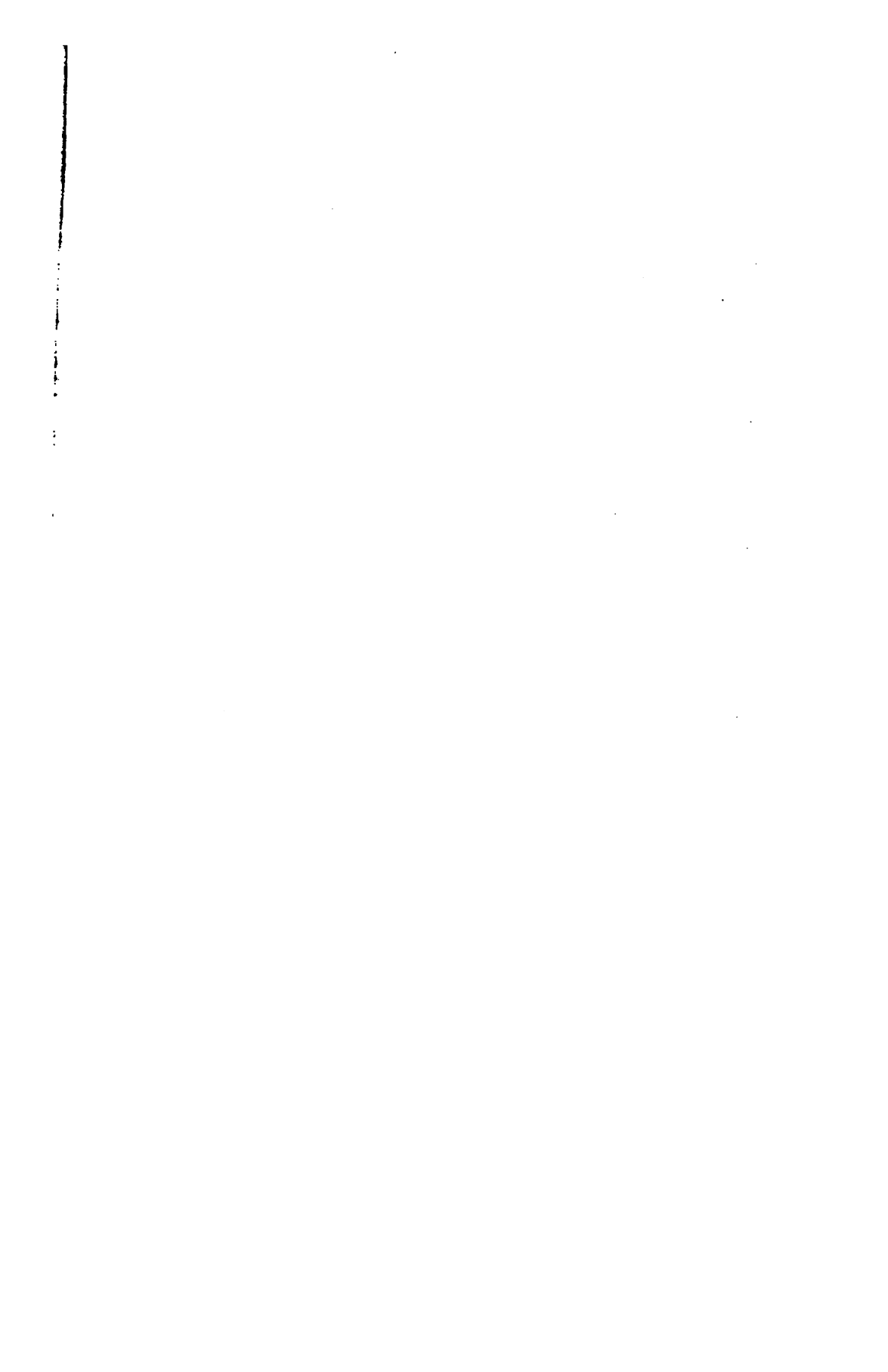


99.



94.





In embryo *C* the same kind of fusion and degeneration as in embryo *B* has occurred, but it has progressed still farther, for the dorsal organs have fused, and also the six pairs of appendages. At the central ends of all the embryos are paired and unpaired ridges, representing abdominal appendages. The dorso-ventral muscles are well developed, and may be seen radiating from the triangular, marginal fold to all three embryos.

FIG. 104, $\times 33$. This extraordinary embryo is a triple one like the preceding. By median fusion and antero-posterior degeneration, each embryo is reduced to a condition like that in embryo *C*, Fig. 103. The dorsal organs and the fifth and sixth thoracic appendages have fused in the median line. Everything anterior to the fourth or fifth segment has disappeared. There is a dark, central area, composed of thickened layers of mesoderm and ectoderm, that represents the anal plates of all three embryos. A series of two or three concentric ridges encircle it, representing the abdominal segments. Between embryos *A* and *B*, and *A* and *C*, are seen the conerescing margins of the mesodermic areas, divided into distinct mesoblastic segments. Nothing of the kind can be seen between embryos *B* and *C*.

The three following embryos belong on the preceding plate, but could not conveniently be placed there.

FIG. 105, $\times 60$, not sectioned. A very flat embryo with inconspicuous, marginal folds. The right chelicera is absent. The embryo is remarkable for the large size of the mouth, and especially for the series of organs arranged along the median line behind the mouth, *a-d*. The first two are transverse depressions, and in the yolk beneath the second depression, as though arising from it, is a collection of cells. The third depression is similar to the second, but with a thick lip, projecting forwards from its posterior border. The fourth is a conical, ectodermic elevation. The abdominal plate is thrown toward the right. The embryo conveys the impression (more strongly than appears in the figure) that it is provided with a series of five mouth-like openings, arranged along the median line.

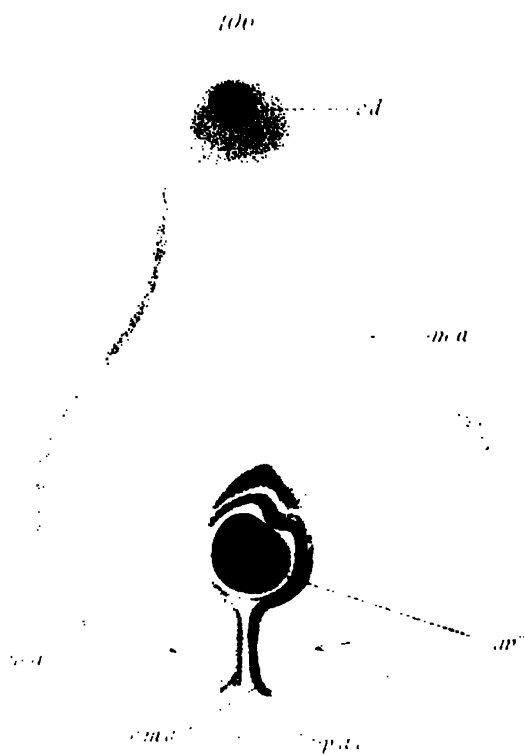
FIG. 106, $\times 60$, sectioned. This embryo is in an advanced stage of development, but extensive degeneration has taken place, leaving but little of the original embryo behind. The cephalic lobes are represented by a dark patch of cells, with a still darker portion, the remnant of the oesophagus, in its centre. In sections, it appears as a slightly thickened layer of ectoderm, with a much thicker mass of mesoderm beneath it. The remainder of the embryo, except the terminal lobe, consists of a single layer of flattened ectoderm cells, with a thin underlying layer of mesoderm. There is no indication of a nervous system, or other ectodermic structures. The margins of the mesodermic area, especially on the side, are thickened, *m.a.*, to form a conspicuous band, composed of several layers of cells. At the posterior end of the body of the embryo is a median, conical projection, probably representing the tip of the abdomen, but possibly a fused pair of thoracic appendages. The appendage rises out of a depression, the anterior wall of which is thrown forward into a shallow pocket.

Back of this papilla, the margins of the mesodermic area have coneresced in the typical manner for stage *D*, and in the yolk beneath the coneresced margins is an oval cloud of degenerating cells, *p.a.c.* The condition of the posterior end of the embryo shows clearly, in spite of its apparently simple condition, the advanced stage of development it has reached. While degeneration seems to be

going on rapidly and extensively in this embryo, there are numerous karyokinetic figures in both the ectoderm and mesoderm at the posterior end of the body.

FIG. 107, $\times 60$. A very small, circular embryo, with a bare trace of cephalic lobes and oesophagus, the latter appearing as a white spot with faint bands on either side, which probably represent the anterior extensions of the nerve-cords.

Two pairs of appendages are present (second and third thoracic?) and back of them are traces of other appendages. The abdominal invagination is small, but quite deep.



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EXPLANATION OF PLATE XI.

The number opposite each figure corresponds to the number of the embryo that was sectioned. The exponent indicates the position and number of the section, and agrees with the number opposite the dotted section line, *s*, in the surface views.

The sections are not intended to show histological details, but merely as a help in the interpretation of the surface views.

FIG. 8^{1, 2}, $\times 200$. Two longitudinal sections of the brain, optic ganglion, and invaginated appendage.

FIG. 9^{2, 5, 7}. Cross-sections of the cephalic lobes, to show the nature of the fold that grows over the brain.

FIG. 10^{1, 2, 3}. Longitudinal sections showing the ganglionic fold over the optic ganglion, and the brain pits.

FIG. 10¹¹, $\times 200$. Longitudinal, vertical section of an invaginated appendage. Same section as 10¹, but shows a part farther back, and more deeply invaginated.

FIG. 11¹, $\times 400$. Section of a marginal vesicle, showing fatty degeneration of the mesoderm cells.

FIG. 20^{1, 2, 3, 4}, $\times 100$. Cross-sections, showing absence of nervous system at the anterior end, and the medianly fused appendages.

FIG. 68^{1, 2}, $\times 100$. Cross-sections, showing invaginated, marginal thickening. *A*. Same more highly magnified, to show the bacteria-like dots on the surface.

FIG. 73^{1, 2}, $\times 100$. Section of a late stage of a degenerate embryo, showing absence of organs and histological specialization.

FIG. 77^{1, 2}, $\times 100$. Section of embryo similar to that in Fig. 66. It shows a remarkably large, horseshoe-shaped body cavity, possibly formed by the fusion of several somites. The cells lining the floor of the cavity are flattened and mingled with fibres, so it has the character of connective tissue seen in very old stages. The somatic mesoderm is on the contrary composed of lymphoid cells, which are here and there wedged in between the inner ends of the columnar, ectodermic ones, as though they arose from the latter by inward proliferation.

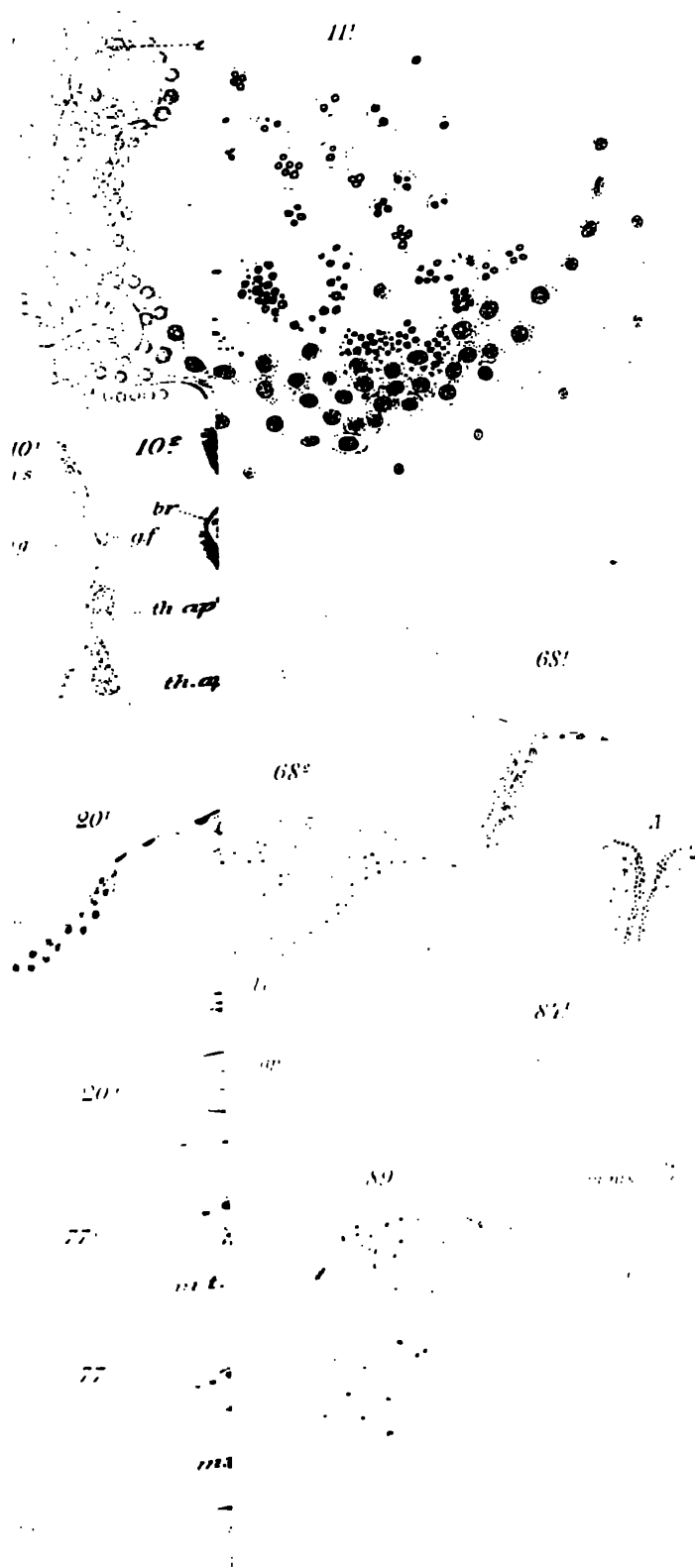
On each side of the cavity is a thickened cord of mesoderm cells, which seems to represent the thickened rim of the mesodermic area. Such a relatively enormous body cavity (?) has not been seen in any other embryos.

FIG. 83^{1, 2, 3}, $\times 100$. Cross-sections of an invaginated embryo, showing absence of recognizable nerve-cords and appendages.

FIG. 84, $\times 100$. Cross-section of an invaginated embryo, showing the last traces of the nerve-cords, appendages, and eyes.

FIG. 85^{1, 2}, $\times 100$. Cross-sections of invaginated embryo, showing the relation of the foldings.

FIG. 89. Section of a very old embryo, reduced to a formless mass of cells. Of the latter, some are multiplying by karyokinesis, others degenerating; some are lymphoid, others finally are the very remarkable amoeboid cells, containing coiled fibres. These cells are found in great numbers in the marginal thickenings of the mesodermic area of very old embryos, and are also found in great numbers in the adult. *B*. Two fibre cells enlarged, showing the fibre lengthwise and in optical cross-section.



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BUDDING IN COMPOUND ASCIDIANS, BASED ON STUDIES ON GOODSIRIA AND PEROPHORA.

W. E. RITTER.

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INTRODUCTION.

THE material that has served as the basis of the paper here presented was collected by myself at various points on the California Coast during the years 1892 and 1893. A considerable part of the investigation has been carried on under

circumstances that have placed me under obligations to several persons and institutions. These I would here acknowledge, and that most gratefully: first of all to Mr. Alexander Agassiz, through whose generosity it was that I was enabled to occupy a table at the Zoölogical Station at Naples for some months during the fall and winter of 1894; next, to Prof. Dohrn and all those associated with him in making the Naples Station the realized ideal of what such an institution should be; and last, but by no means least, to Prof. F. E. Schulze, who so kindly and generously placed the excellent facilities of the Zoölogical Institution of the University of Berlin at my service for some months.

A. GOODSIRIA DURA NOV. SP.

I. MATERIAL. TECHNIQUE.

My specimens were all collected by myself at Santa Barbara, California, during a brief visit there in the last days of December, 1892.

They were all found upon the beach where they had been thrown by the waves; I saw no colonies in their original positions. As they were found in abundance and in a perfectly fresh condition, I conclude the species must be plentiful at this point, and that it lives on the sea bottom not far from the shore. This latter supposition is likewise supported by the fact that most of the colonies are attached either to shore-inhabiting seaweeds, or to *Styela rubra*, an Ascidian very common at Santa Barbara on the piles of the wharf, and on the rocks in shallow water. It is almost certain that the dredge, when brought into use here, will bring it up from its natural abode in quantities. On account of its obvious abundance at this point, I have been somewhat surprised at not finding it elsewhere on our coast; but there is little doubt that further work with dredge and tackle will bring it to light at other places.

As the only killing reagents with which I was provided on my visit to Santa Barbara were picro-sulphuric mixture and

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alcohol, I was limited to these for the preservation of my specimens. Luckily, however, the former proved to be a favorable medium for the purpose. Some of my material has proved to be excellently well preserved, better than any I have been able to get of some other Ascidians by using a large variety of reagents. Some specimens preserved in absolute alcohol were found to be valuable as collateral material.

No opportunity has been afforded me for studying living specimens with any detail, but they are much too dense and opaque to permit of being very satisfactorily studied in this condition. Likewise, since the species belongs to that category of Ascidians in which the mantle clings closely to the test, it is impossible to free the zooids from the colony in preserved specimens so as to study them whole with much satisfaction. One is consequently obliged to depend on the study of small colonies, or small pieces of colonies cleared in oil; on dissections; and on thin sections. The first mentioned method is particularly valuable.

Of the numerous stains employed I have found Paul Mayer's Hæmalum, and Grenacher's Alum Carmine to give the most satisfactory results.

2. DESCRIPTION OF THE SPECIES.

As we are dealing with a new form, it will be necessary to describe it; and this is the more incumbent because it belongs to a group of Ascidians not very well known to science.

As mentioned in my preliminary paper ('94), it belongs to the *Polystyelidae*, a family founded by Herdman ('86). The author has given a good historical résumé of our knowledge of the group, and I may consequently touch this phase of the subject lightly. The six genera of which the family is at present composed were, with one exception, all established between 1843 and the time of Herdman's description of the Challenger collection in 1886. This one exception was added by the author himself. They were described by different writers and assigned by them to different larger groups already known; Carus ('43), for example, regarding the genus described by him

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as being allied to *Clavelina*; while Giard ('74) considered the two founded by him as related to the *Cynthiidae*. Most of the authors believed the particular forms which they studied were true compound Ascidians, though none of them were able to produce the crucial test of this, *viz.*, evidence of the occurrence of multiplication by gemmation. This evidence remained wanting till the publication of my recent preliminary.

By personally studying as many of the known species as were accessible to him, and by critical examination of the descriptions and figures given by other writers, Herdman has amply justified his uniting the genera into a single new family. He has also contributed many valuable observations on the species described by him.

Since the work of this author appeared, there has been, so far as I know, but one other contribution to our knowledge of the group. This consists in the addition by Gottschaldt ('94) of a new species, *Goodsiria borealis*. To this, further reference will have to be made because of its close resemblance to the species now under consideration.

The following is the diagnosis of the new species as my present knowledge enables me to give it:

General Character of the Colonies. Predominating form flat and encrusting. Occurs most frequently on various sea-weeds, and on *Styela rubra*, and often so completely covers these that the outlines of the colonies are determined by those of the particular substrata. But in addition to the flat and encrusting condition, colonies not infrequently occur with fleshy knobs composed of a large mass of test material containing zooids in the entire surface layer but none in the centre.

Colonies from 1 cm. to 5 or 6 cm. in diameter, apparently tending to expand equally in all directions when permitted to do so by form of substratum.

Color in living state, dull red; light brown in preserved specimens.

Zooids. Fully imbedded in the test, not projecting from surface of colony; not arranged in systems, no common atrial orifices; for the most part evenly distributed in surface layer

of test and rather close together. Size of zooids, length 3 to 5 mm., width 2 to 3 mm.

Test. Rather dense, opaque, cells small and not numerous, no bladder cells, slightly fibrillated. Vessels numerous, much branched and anastomosing, terminating, particularly in margins of colony, in many large pear-shaped ampullae; mostly occupying a deeper position in test than the zooids.

Musculature. Not highly developed, fibres not arranged in distinct bands.

Branchial Apparatus. Position of branchial and atrial orifices vary with the character of the colony; when the colony is thin and encrusting they are both placed more dorsally, *i.e.* opposite the endostyle; when the colony is fleshy and massive, the orifices are more anterior and nearer together. No distinct siphons; orifices not lobed, at least not discoverably so in preserved specimens; in some specimens orifices obscurely quadrilateral.

Branchial tentacles simple, usually twenty long and strong ones, and about same number of smaller ones alternating with them. Atrial tentacles present, about twenty in number, much smaller than the branchial. Branchial sac without folds; internal longitudinal vessels rather prominent, 5 on each side, the two dorsal ones on each side nearer together than the others. Small intermediate transverse vessels frequently present. About 12 series of stigmata; 5 or 6 stigmata between each two longitudinal vessels excepting between the two on each side which are closer together, where there are only two or three. Dorsal lamina a plane membrane tending to roll up.

Endocarps. Present in the form of large globular structures attached to the parietal wall of the peribranchial sac, from which they project prominently into the peribranchial space.

Digestive Tract. Situated on left side of branchial sac, distinctly divided into oesophagus, stomach, and intestine. Oesophagus nearly as broad as intestine, and approaching the stomach in length. Stomach somewhat pear-shaped, about 8 deep folds extending lengthwise of the organ, parallel with one another, not converging toward the point of entrance of the oesophagus. Course of intestine, first ventralward from

stomach for a short distance, then changing by a rather wide curve to a nearly dorsal direction which is followed for a distance about equal to the combined length of oesophagus, stomach, and ventrally directed limb of intestine. Anus directed somewhat anteriorly, nearly as broad as any part of intestine, provided with a thick lip. A peculiar thickened band in the wall of the intestine, beginning in the loop, extends in an oblique direction half way to the anus. Lacteal system consists of a prominent coecum projecting from the stomach near its pyloric orifice, into one side of which opens a much smaller tube, the common stem of the greatly branched intestinal part of the system.

Sexual Organs. Both ovaries and testes in the form of "polycarps" attached to the mantle on each side of the endostyle, and projecting into the peribranchial chamber. These few in number and small in size so far as known. Uncertain whether the same zooids produce both ova and sperm or not.

Hypophysis. A simple duct, no branched glandular part, the mouth a simple elliptical opening.

Ganglion. Situated ventrally to the hypophyseal duct, consisting of the usual outer layer of multi- and uni-polar ganglion cells, and an inner cell-less core of nerve fibres.

Budding. Pallial, *i.e.* from the parietal wall of the peribranchial sac.

I have hesitated much in deciding in which of the two genera, *Goodsiria* or *Synstyela*, this species ought to be placed. The only well-marked distinction between them appears to be in the character of the colony, this being designated as massive in the first, and thin and encrusting in the second. Certainly the latter characterization applies to the greater number of colonies of *G. dura* which I have seen, but at the same time it does not apply to all in all their parts. It will surely be allowed by all zoologists familiar with the compound Ascidians that the form of the colony alone is a rather frail peg on which to hang a genus. It appears to me that the difference between a pedunculated and a massive colony is at least as great as that between a massive and an incrusting one. If the latter difference is worthy of being used to separate species into genera,

the former ought to be likewise; but two of the Challenger species of *Goodsiria* described by Herdman, viz., *G. pedunculata* and *G. placenta*, are decidedly pedunculated, while this character is as markedly absent from the other known species. As we shall see presently, if the structure of the *individual zooids* and not the *form of the colonies* is made the basis of comparison, the closest ally to our present species is certainly found in the genus *Goodsiria*, instead of in the genus *Synstyela*.

I have consequently reached the conclusion that while, so far as morphological agreements and disagreements are concerned, my species might be placed with about equal propriety in either genus, since *Goodsiria* is the older of the two (it having been founded by Cunningham in 1872, while *Synstyela* was founded by Giard in 1874) it is more entitled to receive the new comer.

The specific name *dura* I have chosen as applying to the firmness of the colonies due to the density of the testicular matter.

There are three other known species to which this one is closely allied, and with which, consequently it must be compared in some detail in order that its differentiating characters may be brought out.

These are: *Goodsiria coccinea*, Cunningham, *G. borealis*, Gottschaldt, and *Synstyela incrustans*, Herdman. With the first mentioned species it agrees not only in the numerous points of structure common to all species of the family, but also in the form and size of the zooids, points of considerable determinative value for species in this group; but most important of all, *in the absence of folds* in the branchial sac. So exceptional is this condition among the representatives of the family that its occurrence in two species rather closely related in many other particulars may be regarded as evidence of their very close relationship. But that these two forms are specifically distinct there can be no doubt. The differentiating characters are the following. The colonies are much larger and much more distinctly massive in *G. coccinea* than in *G. dura*. According to Herdman ('86), on whose description of the former I base my comparison, the colonies of this species

sometimes reach a length of 46 cm., while I have never seen a colony of *G. dura* more than 10 cm. in length.

The branchial and atrial apertures of *coccinea* are conspicuous and are irregularly four-lobed; in *dura* they are not conspicuous, and in preserved specimens show no trace of lobes. In *G. coccinea* the "meshes" of the branchial sac, *i.e.* the areas of the sac between the internal longitudinal vessels, contain eight stigmata. In *G. dura* the number is not the same in all the meshes, but at the most is less than eight; at the least it is three. But probably the best distinction between the two species is in the character of the stomach. In *G. coccinea* this organ, as described by Herdman, is globular in form, and concerning its folds he says: "There are usually six well-marked folds upon the right side of the stomach. A transverse section shows in addition a single large fold, which projects far into the centre, nearly dividing it into two cavities." Reference to my Figs. 4 and 6, Pl. XII, shows at once that the stomach of *G. dura* is quite different from this. In the first place it is not globular. It is rather schizaster-shaped, if I may be permitted to suppose that the form of this echinoid is any more familiar than that of the stomach which I am comparing with it; but I can think of no other object which it so closely resembles in form as it does some species of this genus. In the *G. dura* stomach there is no one fold that exceeds the others in the extent to which it projects into the chamber.

Another distinction between the two species, that would appear to be constant, consists in the presence in *G. coccinea* of a vessel which, "enclosed in a prolongation of the mantle, leaves the posterior end of each Ascidiozoid and runs for a longer or shorter distance through the test before ending in a dilated bulb." No such vessel occurs in *G. dura*.

Although it is evident from Gottschaldt's description of *G. borealis* that this species and *G. dura* are closely related, it is unfortunately impossible to make as exact a comparison between them as is desirable, owing to the incompleteness of the author's description at one or two critical points. In the form of the colony they appear to agree more closely than either agrees with *G. coccinea*, for the figure representing a

colony of *G. borealis*, shows it to be quite "flat and encrusting," though the author speaks of it as being fleshy. The points in the structure of *G. borealis* which preclude the placing of our California form in that species are these: The four-lobed branchial and atrial orifices; the eight series of stigmata (*G. dura* has twelve); and the presence of folds in the branchial sac. It is stated by Gottschaldt that "zarte Muskelfibrillen" occur in the test; but almost certainly this is an error, the "Fibrillen" which he supposes to be muscle fibres, being similar to those found in the test of many other Ascidians, but which are in all probability correctly considered as not contractile, but mere filamentous differentiations from the matrix of the test. The author also says that the "liver gland" is well developed; but he has in all probability mistaken the lacteal system for a liver, since nothing corresponding to what is generally understood to be the liver in many simple Ascidians is present in at least three closely related species which I have examined with special reference to this point. These species are *Goodsiria coccinea*, *G. dura*, and an undescribed Australian species of *Chorizocormus* kindly furnished me by Prof. Herdman.

I have regarded *Synstyela incrustans* and *Goodsiria dura* as closely related, more on account of resemblances in the form and character of the entire colonies than from similarities in the structure of the zooids. In the former particular, they undoubtedly agree rather closely, but in the latter they are, on the whole, as previously remarked, less closely alike than are *G. coccinea* and *G. dura*. The colonies of *Synstyela incrustans* are said by Herdman to reach a length of 20 cm. in some cases, while it will be remembered that no colony of *G. dura* has been found more than half that size. The individual zooids are also much larger in the former than in the latter species, their length being as great as 8 mm. in the one, and never more than 5 mm. in the other.

The branchial and atrial apertures of *S. incrustans* are described as "conspicuous, but not distinctly lobed." We have seen that in *G. dura* they are not conspicuous, nor are the lobes recognizable at all in preserved specimens, so that in

this particular the last named species agrees about as well and about as little with *G. coccinea* as with *S. incrustans*. But it is when we regard the branchial sacs of the two species now being compared that we find their most important differences. The sac of *Synstyela incrustans* has a rudimentary fold on each side, while it will be remembered that no folds are present in *G. dura*. Unfortunately, Herdman does not tell us the number of internal longitudinal vessels in the sac of either *G. coccinea* or *S. incrustans*; but he has shown eight in a figure of a portion of the sac of the latter, so we are certain that the number here exceeds the number in *G. dura* by *at least three*; of course the excess may be greater, since it is not certain that the eight shown in the figure referred to is the entire number present; in fact it is more likely not to be, since in making such preparations of the sacs as that from which this figure was drawn one does not usually obtain intact the entire width of one side.

In *S. incrustans* there are intermediate vessels, "normally three in number," crossing the meshes of the branchial sac. In *G. dura* I have never seen more than one of these, and frequently this one is absent.

There also appears to be a difference between the two species in the arrangement of the polycarps. In the former they are figured by Herdman as scattered promiscuously over a portion, at least, of the wall of the peribranchial cavity, while in the latter, so far as I have found them present, they are confined to a single row on each side of the endostyle. But great weight cannot be attached to this distinction, since the sexual organs are too poorly developed in all my specimens of *G. dura* to enable one to decide what their arrangement might be in a better-developed condition.

3. THE ZOIDS IN THE COLONY.

This subject is so intimately connected with that of bud-development that it must be treated somewhat more fully than have been the other points of adult structure. I cannot discover any constant arrangement of the Ascidiozooids in the

colonies. Fig. 1, Pl. XII, represents a colony, natural size, growing on a laminaria leaf. As here seen, the zooids are, as compared with many compound Ascidians, rather distant from one another. They are very regularly distributed, there being no suggestion of systems. Neither have the zooids any constant relation either to the colony as a whole or to one another, with reference to their antero-posterior axes; though in general where the colonies are narrow and elongated the longer axes of the zooids correspond to the longer axes of the colonies. The arrangement of the adult zooids is of course determined by the relations which the developing buds hold to their parents, and in this there appears to be no constancy beyond the fact that the buds are generally confined to the borders of the colonies. I say generally this is the case; but it is not without exception, for in several instances I have found young blastozooids so situated that older ones intervened between them and the edge of the colony. (Fig. 2, Pl. XII.)

The question of the relation of the buds to the older zooids is important because it obviously involves the questions whether all the zooids of a colony are capable of producing buds, and at what age in the life of the zooids their buds are produced. And this last question leads back again to the fundamental one of whether or not the cells that initiate the bud-development are derived as unmodified or embryonal cells from the parent, the grandparent, and so on to the sexually produced embryo that was the common ancestor of the colony. What I have to say on this point will be better reserved until I speak in detail of the *Anlage* of the bud, my purpose here being to describe merely the form and composition of the colony as a whole. In a few instances (Fig. 2, buds *a* and *b*), for example, there is a suggestion that at the borders of the colonies the younger buds occupy positions alternating with the next older ones, but a little in advance of them toward the edge of the colonies. If, however, such a law of arrangement exists, it prevails less frequently than do the exceptions to it.

To Metchnikoff's ('69) denial that buds are produced by the vessels in Botryllus, Giard ('72) raises the objection that if this

were true it would be impossible to explain "la production d'étoiles multiples et distantes dans le cormus d'un Botryllien." The remoteness of the young buds from any older zooids in *Goodsiria* has likewise frequently proved a stumbling-block to me in seeing how they could in such cases have been produced in the usual way, *i.e.* from the wall of the peribranchial sac. But I have given much attention to the point, and am quite convinced that in reality this is their only source. Herdman ('86) expresses the opinion that the ampullae of the vessels will be found to give origin to the buds, but such is apparently not the case here any more than it is in *Botryllus*.

The frequent remoteness of the buds from their parents must be due to their having grown away from the latter before they become fully severed from them. From the firmness of the test and the character of the young buds I can hardly believe that they have any power of independent migration throughout the test.

This conjecture, that the remoteness of the buds from their parents is due to the growth of the bud before it becomes severed, harmonizes with the view that pallial budding as it takes place in *Botryllus* and *Goodsiria* is not of necessity fundamentally different from the stolonian method of budding. The comparison between these two methods becomes still more interesting from the discovery that in *Perophora* the buds are connected with the septum of the stolon by their peribranchial instead of their branchial sacs. But I shall discuss this point further after having described in detail the development of the bud in each species.

There is certainly no septum in the vessels of the test, (Figs. 3, 5, 7, etc., Pl. XII, *ec. ves.*); and if buds were to be produced in connection with them, it would have to be by a method essentially different from any of the generally recognized types of gemmation among Tunicates. Since the blood of the zooids passes freely into the testicular vessels, and since the young sexual cells float in the blood, it is not beyond the range of possibility that these cells may pass into the ampullae of the vessels, and there form themselves into the "inner vesicle," the all important precursor of the blastozooid.

It is an interesting fact that Herdman ('86, p. 90 *et seq.*) has observed a process in *Colella pedunculata* which would appear to be a realization of that here imagined. As the author himself says, his observations are rather fragmentary, and consequently his account is much less full than we might wish it to be.

That the buds arise in this anomalous manner in this genus, he, however, seems to be convinced, and his description and figures undoubtedly furnish good ground for his conviction. It is certainly very much to be hoped that opportunity will before long be afforded some zoölogist to study the subject more fully. In a few instances I have found a massing of cells within the ampullae that is strongly suggestive of the process described by Herdman. Minute examination of these aggregates has, however, failed to furnish any evidence that they produce buds. Two such cases are shown in Fig. 7, Pl. XII. They are quite conspicuous when cleared in oil and examined with a low magnification.

The vessels generally occupy a deeper position in the test than do the zooids, so that the test surrounding the zooids is less thickly penetrated with them than are its deeper portions. This is shown in Fig. 5, Pl. XII.

It has been mentioned above that the buds become fully severed from the parent zooids at an early stage in development. This fact raises the question of the extent to which the zooids in this species are independent of one another. It is certain that many of them are in connection with the vessels of the test for at least a portion of their lives, and are consequently in vital connection with one another. But this connection is almost certainly secondary, and, I believe, not essential to the development of the zooids.

As the young buds become larger, they press more and more closely against the vessels that were in contact with them, or nearly so, at the beginning, and by this pressure a fusion of the vessel wall with the outer or ectodermal wall of the bud is produced, and then later a perforation of the fused walls occurs, and the lumen of the vessel is thus brought into communication with the body space of the zooid.

Figs. 37 and 38, Pl. XV, taken from two different zooids, represent the points at which the vessels open into the body space of the zooids. I would call particular attention to Fig. 37, since this illustrates a much more common appearance than that shown in Fig. 38. The projections, *ec.ves'*, into the cavity I understand to be due to the vessel's having pushed itself therein, either by its own growth or by its passive resistance to the expansion of the developing zooid. In many cases it appears that the wall of the zooid has been pushed in to a considerable extent by the vessel; and by an interfolding of the vessel wall and the zooid wall, the character of the connection has become quite complicated. In some instances these projections extend entirely across the body space to the wall of the peribranchial sac.

I said above that it is *almost certain* that these communications are secondary. This qualification was made because one might ask if the vessels do not sprout out from each of the blastozooids, as it would seem they must have done from the sexually produced ancestor of the colony.

That this process never occurs I cannot positively affirm, but I have no evidence that it does, and two considerations incline me to the belief that it does not. In the first place, the peculiar character of the connection, as described and shown in Fig. 37, seems to me to speak against such a process; and in the second place, one would suppose that if it takes place at all it would do so at a rather early stage in the development of the zooid, before the ectodermal cells in the region from which the vessels would have to be produced had undergone modification. But I have searched in vain for cases of such formation.

By carefully examining all the sections of numerous buds at various stages in development, one can determine that there are no connections whatever between them and the vessels. This is the fact that makes me believe that the connection of the buds to the vessels is not essential to the development and life of the zooids.

4. DEVELOPMENT OF THE UNDIFFERENTIATED BUD.

The earliest stage which I have observed in the development of a bud is represented by Fig. 11, Pl. XII. Here the bud *Anlage* is clearly marked, *bd.a.*, both by the character of the cells and by the slight evagination already seen. But as yet the ectoderm shows no indication of having been affected by the process which has begun in the wall of the peribranchial sac beneath it. It is true its cells are higher immediately over the evagination than they are at any point more ventralward; but they are not higher than they are from this point upward toward the dorsal side of the zooid, as the figure shows. That is to say, the ectoderm cells are not different over the bud from what they are in a corresponding position of a zooid in which no bud is developing.

The figure shows approximately the stage of development of the parent zooid. The endostyle, *end.*, is not yet fully differentiated, histologically, and the thickened places, *st.a.*, in the wall of the peribranchial sac opposite the bud show where branchial stigmata are going to form. The string of cells, *en.c.*, near the bud, is a fragment of one of the "endocarps" that has been broken and displaced somewhat in the section cutting. It consequently has no significance.

To the important question of whether the bud *Anlage* arises from a "budding zone" on the peribranchial wall in which the cells are "embryonal," and are endowed from their very origin with peculiar bud-producing powers, my observations do not enable me to give a wholly satisfactory answer. However, certain facts are suggestive in this connection. I have never found anything that appears like a "budding zone" similar to that described by Oka, for example, in *Botryllus*, and I find that the walls of the peribranchial sacs in the developing bud are, after the very first stages of their growth, very thin throughout, the cells being considerably flattened. Their development is, of necessity, accompanied by cell division, but I am unable to discover that this is more noticeable in one region than in another; or that the cells have a different appearance or distribution in one locality from what they have in another.

One of two conclusions seems to be inevitable from the facts observed: *Either there are no budding zones containing specially endowed cells; or a large majority of zooids are incapable of producing buds.*

It is true that a comparatively small number of zooids with buds actually in connection with them have been found; but I suppose this to be due generally to the early stage at which the buds are severed from their parents, rather than to the incapacity of the zooids to produce buds. But I would by no means assert that all zooids are capable of asexual reproduction. Later stages in the development of buds, though before severance from their parents, are shown in Figs. 9 and 10, Pl. XII. and Figs. 12-15, Pl. XIII. Fig. 9, Pl. XII, represents a zooid with its bud, seen as a transparent object, but not sufficiently cleared to render distinctly visible the several organs and layers. On the other hand, Fig. 10 was drawn from a specimen well cleared in cedar oil, and consequently so transparent as to make the optical section at the level represented almost as distinct in its various parts as an actual section in the same plane would be. These figures explain themselves sufficiently. The position of the buds far forward on the zooids will be noticed in all these cases. This appears to be their usual position. A transverse section of a bud of a stage about corresponding to that shown in Fig. 10 is presented in Fig. 14. This represents the tenth section from the tip of the bud. The thickness and irregularity of the inner vesicle are here conspicuous. The sections of the same series near the parent zooid show the inner vesicle to be here considerably thinner than it is in the section figured. The difference in thickness in the two regions is, I suppose, due to the fact that growth is taking place chiefly toward the end of the bud. The thinner, basal part is where, a little later, the endoderm will become disorganized in course of the cutting off of the bud from the parent.

The question may arise here if the unequal thickness of the endoderm at various points, as shown in Fig. 10, is not an indication that the differentiation of the organs has begun at this stage.

This does not seem to be the case. At any rate, as we shall presently see, at a still more advanced stage, *i.e.* after the bud has become fully severed, the wall of the inner vesicle is, in some buds at least, still entirely undifferentiated.

Fig. 15, Pl. XIII, shows a section of a bud that is very nearly severed from its parent; in fact, the severance is practically complete, there being a mere neck of scattered cells, *v.c.*, marking the former connection between bud and parent. It is here seen that the ectoderm of the zooid is not fully closed together at the point where the bud was cut away. Here the inner vesicle is wholly undifferentiated, it presenting in every section the same appearance as that shown in the one figured; and it should be said that buds occur in which every trace of the connecting strand has disappeared, but in which the inner vesicle still retains its simple, unmodified condition. However, it does not appear to remain long in this state.

In describing the further growth of the bud it will be best to follow the course of development of each organ separately, but before beginning the description I wish to call attention to the interesting fact that *in different buds the order of appearance and stages in development of the several organs are subject to considerable variation with reference to one another.* I will point out specific instances of this as I proceed with the description.

5. DEVELOPMENT OF THE ORGANS.

a. *The Branchial and Peribranchial Sacs.*

I have been unable to make my observations on entire buds and on sections agree exactly regarding the initial step in the formation of these structures. By examining whole buds the impression is gained that the process is begun by the growth of two folds which will ultimately form partitions separating the primitive simple vesicle into three portions — two lateral ones, the peribranchial sacs, and a middle one, the branchial sac. These folds are seen at *p.f.*, Fig. 17, Pl. XIII, in which their formation is already advanced to a considerable extent; but in Fig. 16 the slight angles marked by the same

letters are the very beginnings of the folds. In neither of these figures, nor in fact in any of the numerous similar ones that might be given, does it appear as if the peribranchial sacs begin as *evaginations from the primitive vesicle*. It does not appear here as if the peribranchial sacs are initiated by the active growth of the portions of the primitive vesicle walls which are to enter into them, but, as already said, by the growth of the partitioning folds. On the other hand, sections of a very early stage in the development of the sacs show that in one instance, at least, there takes place an active growth of the *Anlage* of the sacs themselves, resulting in *well-defined evaginations*. (See Figs. 20 and 21, *br.s.a.*, Pl. XIII.) These figures are drawn from sections of a bud presenting an earlier stage in the development of the sacs than that represented by Fig. 16, and so it might be assumed that the sacs of the latter individual had their beginnings in such evaginations as those shown in Figs. 20 and 21, even though all indications of them are by this stage completely lost.¹

But if I am right in supposing the angles *p.f.* in Fig. 16 are the beginnings of the folds, it is not quite clear how such a condition as that presented by this bud would be related to one in which the well-defined evaginations of Figs. 20 and 21 were present. The only way of harmonizing the two conditions, so far as I can see, is to suppose that the sacs begin with the evaginations, and that these grow rapidly in width, but very little in depth, till they extend over nearly the whole of the sides of the primitive vesicle. Posteriorly they extend ultimately so far as to be separated only by the angle in which the intestine (*int.*, Fig. 16) develops, and anteriorly the angles are almost obliterated, but the sacs do not approach each other so closely as behind, but remain separated by the thickened area (*end.*, Figs. 16 and 17), which will give rise to the endostyle. Thus we should have the condition presented by Fig. 16 as a more advanced stage of development derived directly from the earlier distinctly evaginated stage. And the fact that the hypophyseal duct and intestine are both begun in the bud

¹ I have since seen in a whole bud an evagination from the inner vesicle, at least on one side, that in all probability corresponds to *br.s.a.* in Figs. 20 and 21.

represented by Fig. 16, while neither is yet indicated in the bud with the peribranchial evaginations, also suggests that the former is older than the latter. But the discrepancy between the two conditions may be explained in another way; it may be due to individual variation; in any case, however, it cannot be a matter of fundamental importance.

The initial steps in the formation of the peribranchial sacs accomplished, the remainder of their development is followed without difficulty. The sickle-shaped partitioning folds, *p.f.*, Fig. 17, extend their arms, one on the dorsal, the other on the ventral side of the inner vesicle, farther and farther backward. But of the two arms of each fold, the ventral one takes by far the more important part in effecting the ultimate complete separation of the peribranchial from the branchial sacs.

The process is clearly illustrated by Figs. 16-19, Pl. XIII, representing dorsal views of whole buds examined as transparent objects; and by Figs. 22-24, Pl. XIII, and Figs. 26-29, Pl. XIV, inclusive. The series of transverse sections represented by Figs. 22-24 are from a bud in a stage of development slightly more advanced than that shown in Fig. 17, Pl. XIII. Fig. 22 is the most anterior of the three sections drawn, and, as will be seen, passes through the point at which the branchial siphon, *br.sip.*, is being formed. Fig. 23 is fourteen sections farther back. It shows both the dorsal and ventral arms of the sickle-shaped folds, *d.f.* and *v.f.*, those of the right side (left side of the figure) very nearly meeting each other, and so making the separation of the right branchial sac at this point nearly complete. Fig. 24 represents a section farther back, and shows at this position merely a trace of the folds, the three sacs of the anterior region being here merged into one common cavity representing the unmodified remnant of the primitive inner vesicle. Figs. 26-29, Pl. XIV, represent similar sections of a still older bud. That shown by Fig. 26 is most anterior, and passes through the opening of the hypophyseal duct into the branchial sac, *hy.m.*, and hence is slightly farther back than the section shown in Fig. 22 of the preceding series. Fig. 28 represents a section sixteen sections farther back. It is seen that the dorsal folds take no part in the formation of

the peribranchial sacs in this region. Eight sections farther back, Fig. 29, the ventral folds no longer appear, and we have again an unpartitioned portion of the primitive vesicle.

From the figures of the whole buds, and also from those of the series of sections, it will be seen that the folds do not extend back parallel to each other, neither do they occupy a vertical position. They converge both posteriorly and ventrally, so that the middle or branchial sac is made cone-shaped, the apex of the cone being directed backwards. This becomes still more distinct at a later stage in development (see Figs. 18 and 19, Pl. XIII). An inclination toward the left side of the posterior extremity of the cone is now noticeable, and this becomes more pronounced at a later time (compare Figures mentioned). In some buds it is much more prominent than in others.

We may now pass to the practically completed condition of the branchial apparatus. A dorsal view of an entire bud in such a stage is shown in Fig. 19. The only changes that will take place between this and the fully adult condition will be some unimportant ones, so far as our present purpose is concerned, in the relations of some of the parts to one another, and a great increase in size of all the parts. From the preceding stage the most important changes have perhaps been those taking place in the posterior region of the animal.

Recurrence to Figs. 23 and 24, Pl. XIII, will recall the fact that at this stage the three sacs opened widely into a common chamber in the posterior part of the bud. In the stage we are now considering such is no longer the case. The two ventral folds by growing up to and fusing with the dorsal wall of the primitive vesicle for some distance, and then by fusing with *each other* but *not* with the *dorsal wall*, for the rest of the way back to their extremities have effected the complete separation of the branchial sac from the peribranchial sacs. These relations will be easily understood by comparing Fig. 24 of the preceding stage with Fig. 28 of the present one, and then Figs. 30 and 31, Pl. XIV, with Fig. 28. Figs. 30 and 31 are from a bud considerably more advanced than the one represented by Figs. 26-29. Fig. 31 shows at *br.s.* the posterior tip of the

branchial sac. One or two sections farther back this cavity disappears, and to the very last is wholly shut off from the large single surrounding cavity marked *at*. The section shown in Fig. 31 is thirteen sections farther back than the one shown in Fig. 30.

It thus appears that while the peribranchial sacs become wholly separated from the branchial sac, they do not become separated from each other, but remain in very wide communication, this communication occurring both behind the posterior extremity of the branchial sac and over its dorsal part. (Compare Fig. 31.) It is this common cavity into which the two peribranchial sacs open, and which may be regarded as an unmodified remnant of the primitive inner vesicle that has been called the cloaca ; but it is important to recognize that in this species, as in many others, it is neither morphologically nor physiologically wholly distinct from the peribranchial sacs. By this time numerous branchial stigmata have formed, particularly in the anterior portion of the branchial sac. The peripharyngeal band and the branchial tentacles are already partly developed, and the dorsal lamina, *d.l.*, Fig. 30, has become quite a conspicuous object on the sections, though it has not yet become rolled over at its edge as it always appears in the adult (Fig. 6, Pl. XII). The endostyle is also distinctly marked out, though it is not histologically fully differentiated, (*end.*, Fig. 30). Likewise the internal longitudinal bars (*i.l.b.*, 1, *i.l.b.*, 2, etc., Fig. 6) have begun to form.

The formation of the branchial and atrial openings seems deserving of a little special attention. The development is initiated by an evagination from the inner sac, for each orifice (Fig. 35, *at.sip.*, Pl. XIV). A little later an invagination forms immediately over this in the ectoderm ; the two meet, fuse, the fused wall becomes broken down, and by gradual but later-accomplished perforation of the overlying test, communication is established between the outside world and the respective sacs (Fig. 36, Pl. XV). The fact to which I wish to call attention appears in the course of later development. It is illustrated by Fig. 36, drawn from a section through a branchial siphon in which the opening is completed so far as the cellular

walls taking part in its formation are concerned. The overlying test is not yet perforated, however. The great growth of the epithelium lining the newly formed siphon, resulting in its remarkable thickness, and the wide, circular double folds, *s.ec.*, *s.f.*, and *s.f.*¹, is the fact to which I refer. Study of various stages in this growth shows that all the thickened part of the epithelium is derived from the ectodermal invagination, and that the formation of the folds is due to growth of this ectodermal epithelium. The secondary fold, *s.f.*¹, represents the evagination from the inner layer, it remaining passive, while the other portion grows rapidly, and produces, as the figure shows, a well-marked valve. As development goes on the whole siphon expands, and this fold or valve becomes narrower and narrower, and finally in the adult it is wholly obliterated, at least in the normal uncontracted condition of the animal. The formation of this fold is probably to permit of the protrusion of the siphon above the general level of the colony in its fully developed state. As shown by this figure, at this stage, *i.e.* previous to the perforation of the test, there is no such protrusion. This explanation hardly accounts, however, for the great height of the entire lining epithelium during these stages of development.

A very different explanation suggests itself, though one too imaginative to be deserving of more than a mere mention. One might conjecture that as incident to the abbreviated development of the Ascidian blastozoid as compared with the embryonic development, the earliest, paired stage in the origin of the atrial opening, so well known in the larval history of many Ascidians, had been entirely lost in the development of the bud, and that the peculiar conditions above described represent the stage in the formation of that opening immediately after the fusion of the two primitive paired embryonic orifices. Van Beneden et Julin ('84), pp. 625-627, to whom we are indebted for the facts in the embryonal development above referred to, quite insist on the importance of distinguishing the cloaca proper from the peribranchial cavities. They point out that in *Phallusia scabroïdes* the cloaca is *wholly* lined by an ingrowth of ectoderm from the dorsal side

of the larva between the two atrial orifices ; while, on the other hand, "les cavités péribranchiales sont . . . delimitées en dehors *seulement* par l'épiblaste," and even this partial ectodermal lining arises independently, both in time and position, of that which lines the cloaca.

If there is any value in the suggestion here made that the ectodermal fold, *s.f.*, Fig. 36, is comparable to the true cloaca of the above-quoted authors, it would not be lessened by the rejection of their distinction between cloaca and peribranchial cavity in so far as this distinction rests upon their view that a portion of the peribranchial epithelium is of endodermal origin.

But, as already said, I do not regard the suggestion, with the only facts that I now have to base it on, as deserving more than a mere mention.

b. *The Digestive Tract and its Appendages.*

The *Anlage* of the intestine is established at a very early stage in the development of the bud. It appears at a point on the primitive inner vesicle that is ventral, posterior, and slightly to the left.

In the earliest condition seen it is a mere short, simple projection growing from the wall of the vesicle, situated in a notch, or indentation of its side in the region mentioned. Fig. 16, Pl. XIII, representing a dorsal view of a whole bud, illustrates the above statements, but it must be borne in mind that the bud is seen as a transparent object, and that the hypophyseal and intestinal *Anlagen* would not, as represented, be seen at the same level. The notch above mentioned is clearly seen in this figure as far as the posterior side of the vesicle is concerned ; but sections from similar buds enable one to see that it is quite as well marked on the ventral side as it here appears on the posterior side. Concerning this notch, or rather the parts of the vesicle adjacent to it, I shall speak further in dealing with the development of the heart ; I shall, therefore, leave the subject for the present after mentioning that at *no time in the life of the zooid does the digestive tract*

extend so far posteriorly as do the backward extensions of the vesicle.

During its early stages of development the intestine projects downward and to the left from the vesicle to which it is attached, so as to lie for the greater part in the wide body space that surrounds the vesicle (Fig. 29, *int.*, Pl. XIV). As development advances, however, it becomes pushed into the vesicle, so that ultimately it lies wholly within it, *i.e.* within the atrium in the adult, and by carrying the wall before it as it enters the vesicle, it comes ultimately to be wholly enveloped by a thin layer of epithelium, which is attached through a thin double-layered mesentery to the epithelial lining of the atrium. Figs. 29, 30, 32-34, Pl. XIV, illustrate these statements. Fig. 29 is from a section that passes through the oesophagus, *oe.*, and at the same time cuts the intestine, *int.*, in its widest part at this stage.

At this time the intestine lies, as mentioned above, in a notch in the ventral side of the vesicle, but projects largely into the body space. It has as yet grown very little in length, but its lip is already directed forward where it appears in the fourth section in front of the one here figured. The distal part is considerably smaller in diameter than the proximal, the stomach and intestine proper being thus distinguished from each other even at this early period.

The organ grows both forward and outward with advancing development, and very soon begins to take on the curved form so characteristic of the Ascidian digestive tract. The curvature is produced by the distal end becoming directed at first outward and dorsalward, and then a little later backward. The plane of the loop stands at first at an angle of about 45° to the horizontal plane. Owing to the backward direction of the tip, a section tangent to the loop first appears in a series cut from before backward; and then a few sections farther back the loop is wholly passed and a double section of the organ is made. This condition is shown in Fig. 32, *res.* being the rectum, and *st.* the stomach. The long pouch-like appendage from the stomach, *l. coe.*, is the beginning of the lacteal coecum and duct. It is noteworthy that at this stage it is almost as large

as the intestine proper. By this time the pushing of the organ into the vesicle, as mentioned above, has advanced considerably (Fig. 32). With further growth the rectum becomes directed inward toward the branchial sac, as well as backward and dorsalward, so that, in the nearly adult state, transverse sections of the animal, which pass through the anal opening, already formed at a considerably earlier time, cut the rectum lengthwise for some distance, at the same time that they pass transversely through the stomach (Fig. 6, *an., rec.*, and *st.*, Pl. XII). It will be observed in this figure how fully the whole digestive tract has now come to lie in the atrium and left peribranchial sac. The rectum reaches across the chamber, and comes in contact with the wall of the branchial sac, and actually forms a secondary attachment to it, so that a rectal mesentery is produced (Fig. 34, *mes.rec.*, Pl. XIV). In the section this mesentery does not appear, but it does in sections a little farther back, none of which, however, would illustrate some other points as well as does this one; consequently, I have chosen this for the figure, and marked the position of the mesentery diagrammatically. The original mesentery (Figs. 33 and 34, *mes.gas.*, Pl. XIV) is in the adult attached to the parietal portion of the atrial epithelium well out on its side, *i.e.* quite remote from the median ventral line, and extends across as a rather narrow band to the digestive tract in the region of the stomach and lacteal coecum (Fig. 34). The differentiation of the lacteal system into the coecum, the duct, and the ramifying portions, as we have seen them to exist in the adult animal, has been fully accomplished by the time the stage we are now considering is reached. The same is true of the formation of the longitudinal folds of the stomach, and the peculiarly modified longitudinal band in the wall of the intestine.

A secondary attachment of the rectum to the wall of the peribranchial sac is said by Hjort ('93), p. 596, to take place also in *Botryllus*.

c. *The Pericardium and Heart.*

The origin of the common *Anlage* of these two structures has been the subject of almost as much diversity of statement for different groups of Tunicates as has been the origin of the nerve ganglion. It is, therefore, particularly satisfactory to find that in this species its method of origin is so clear as to leave no doubt about what it is.

At the time of writing my preliminary communication I had not succeeded in finding the earliest stage in the development of the structure ; further search, however, since then has borne the desired fruit.

It arises from the postero-ventral wall of the inner vesicle, but does not become fully severed until the ventral folds which play the major part in separating the branchial from the peribranchial sacs have extended nearly back to the intestinal *Anlage*. These folds, consequently, enable us to locate more precisely the point of its origin.

Figs. 39-41, Pl. XV, are from a series of transverse sections of a bud in a stage of development considerably younger than that shown in Fig. 18, Pl. XIII. Fig. 39 represents the tenth section in front of the one from which Fig. 40, passing through the pericardial *Anlage*, *pc.a.*, is drawn. The ventrally directed pouch-like fold, *r.pb.s.*¹, seen in Fig. 39, is a portion of the right peribranchial sac, and it is at the posterior extremity of this that the pericardial *Anlage* originates. As shown most clearly in Fig. 41, it is an evagination from the inner vesicle, but, as just stated, is at the point reached at this stage by the posterior extremity of the right peribranchial sac. *r.v.f.*, in Figs. 40 and 41, indicates the right ventral partitioning fold. The *Anlage* of the pericardium appears to become separated from the vesicle at its anterior end first, then later at its posterior end. A later stage, slightly after its complete separation, but while it is still a simple small vesicle, is shown in Fig. 42, *pc.v.*

According to Oka ('92), p. 535, the pericardium in *Botryllus* arises as "eine solide Wucherung des inneren Blattes der

ursprünglichen Knospe ; aber da die betreffende Stelle gerade im Winkel zwischen der mittleren Blase und der Anlage des linkern Peribranchialsackes liegt, ist es schwer zu entscheiden, ob sie speciell der ersteren oder der letzteren entstammt."

Hjort also ('93), p. 602, found the pericardium of the *Botryllus* bud to appear first as a solid cell mass ; but concerning the origin of this mass, whether from the "endoderm" or from mesoderm cells he was not able to decide.

Pizon ('93), p. 44, on the other hand, describes it as arising in this genus as a small diverticulum, and neither from the branchial nor the peribranchial sacs, but from the primitive vesicle.

But whether it begins as a solid cell mass or as a diverticulum signifies very little ; and of almost as little significance is the question whether it arises from the primitive vesicle, from the branchial sac, or from the peribranchial sac, since its point of origin is at this stage of development an indifferent point as regards the three structures mentioned. It is not at all impossible that absolute exactness in observation would find it to arise from the branchial sac in some individuals, from the peribranchial sac in others, and from the primitive vesicle in others. Indeed, the observed facts in relation to variations in the time and position of appearance of different organs make such a conjecture rather probable.

The only fundamental question raised is as to the possibility of a mesodermal instead of an endodermal origin. This question, and also the question of the epicardium, I shall discuss after having presented my observations on the development of the organ in *Perophora*. The formation of the heart by an invagination of the pericardial vesicle is here so entirely similar to what is well known in many other Ascidians that a detailed description of the process would be superfluous. A mere reference to Figs. 29 and 30, Pl. XIV, in which two stages in the development are incidentally shown, will suffice.

d. *The Hypophysis and Ganglion.*

a *The Ganglio-hypophyseal Duct.*—The hypophysis always arises very early, in some buds its origin being the first interruption of the simple spherical form of the primitive inner vesicle. This is the case, for example, in the bud a section of which is represented in Fig. 43, Pl. XV. Its method of origin can be very well seen by examining with a low magnifying power a whole bud from its dorsal side, the bud having been first cleared in oil. A drawing of such a bud is shown in Fig. 16, Pl. XIII, where *hy.a.* is the *Anlage* of the organ now under consideration. By observing it under a constantly changing focus, one finds that it is a groove-like evagination from the dorsal wall of the inner vesicle. A transverse section of a bud in the same stage of development, and cutting the evagination, is presented in Fig. 43. The groove is very simple and is quite uniform throughout its length.

The same section is shown at *bd.*, in Fig. 5, Pl. XII, where the clearly seen orientation of the bud in the colony, and the position of the evagination on the dorsal side of the bud, leave no room for doubt that the evagination is the beginning of the hypophysis. The *Anlage* remains in this groove-like condition only a short time; for in a stage of development of the bud only a little more advanced than that shown in the two figures to which attention has just been directed, it appears as a tube wholly separated from the vesicle, except at its anterior end, where its lumen communicates with the cavity of the vesicle. This communication remains throughout the life of the animal as the mouth of the hypophysis. As the tube becomes constricted off from the wall of the vesicle, it terminates posteriorly as a simple blind pouch, and does not at any stage communicate with either of the peribranchial sacs, as is agreed by Oka, Hjort, and Pizon to be the case in *Botryllus*. Hjort ('95) also shows such a communication in *Glossophorum sabulosum*. But as remarked by Hjort and Bonnevie ('95), p. 392, this communication can have very little significance. They base this remark on the fact that it does not take place in the buds of *Distaplia magnilarva*, studied by them, while it does in the

buds of genera no farther removed from this genus than are *Botryllus* and *Polyclinum*. I agree that the fact has very little significance, but would point to a different reason for so regarding it. The posterior communication is always transient and without physiological importance. The hypophyseal duct forms from the dorsal wall of the inner vesicle at an early stage of development of the bud, before the limits of the peribranchial sacs are sharply established; and since their limits on the dorsum of the vesicle are always very close to the posterior extremity of the duct, a slight variation in the relative position of the point at which the duct terminates, and the course of the partitioning folds of the peribranchial sacs; or of the time of closure of the duct opening; or the formation of the folds, would determine whether the posterior opening of the duct should be into the primitive inner vesicle, the branchial sac, or one or the other of the peribranchial sacs, or into the atrium. I shall show presently that there is a brief period in the history of the duct in *Goodsiria* when it also has both an anterior and a posterior opening. The posterior opening closes, however, at a stage so early in the development of the peribranchial sacs, that we can only regard it as pertaining to the primitive vesicle itself.

As regards the later history of the duct as such, little more need be said. In the nearly adult state its dorsal wall, Fig. 50, Pl. XV, is exceedingly thin, it being but one layer of cells thick, and the cells of this one layer are considerably flattened, and their nuclei are far apart. The ventral wall, on the contrary, remains, at least in the oldest stages of which I have made sections, several cells thick. Attention may here be called to the entire absence of the hypophyseal gland.

The formation of the duct as I have here described it differs in some unimportant particulars from its formation in *Botryllus*, where, according to Hjort and Pizon, it is not at its beginning a groove, but an evagination of about equal diameters transversely and longitudinally. This evagination then grows out into a forwardly directed pouch, the blind tip of which fuses later with the wall of the branchial sac, and its lumen gains communication with this sac by a perforation at the

point of fusion. The permanent hypophysis mouth is therefore in this genus secondary, and not primary as in *Goodsiria*. The groove-like *Anlage* of the duct in the latter genus is apparently very similar to that which Kowalevsky ('74a), p. 450, has described in *Didemnum styliferum*, the bud-development of which resembles that of *Goodsiria* in some other respects.

β *The Ganglion*.—The three papers of Oka, Hjort, and Pizon, on budding in *Botryllus*, reference to which has already been made, were written so nearly simultaneously that neither author was acquainted with the result of the others' work while prosecuting his own. *No two of these investigators agree concerning the origin of the ganglion*, though each was duly impressed with the theoretical importance of the question, and was also familiar with the discordance in the previous results obtained by several investigators who had studied the blastogenesis in various other Ascidians. This fact testifies convincingly to the difficulties involved in the subject. To these difficulties I too can bear witness, and while doing so may be permitted to say that I have striven hard to search out every fact that might bear on the question, and to draw my conclusions uninfluenced by any bias in favor of one or another of the several prevailing views. Four different origins have been found for the ganglion in the buds of different compound Ascidians by different students. 1. It has been derived directly from the central nervous system of the parent zooid. 2. It has been derived from the free so-called mesoderm cells of the body space. 3. It has been derived from the ectodermic, or outer vesicle of the bud. 4. It has been derived from the inner primitive vesicle, or, more precisely, but what is the same thing, from the hypophyseal duct. The first was advanced by Pizon ('93), for *Botryllus*, evidently suggested by the method of origin of the ganglion in the buds of salpa. But as the author does not pretend to have proven such a process to take place here, and as the facts on which he bases his belief are exceedingly meagre, this may be dismissed without further remark.

The suggestion of a mesodermal origin was, so far as I know, first made by Seeliger in his studies on *Clavelina*. It

was, however, based on indirect and theoretical grounds, viz. : on the facts that in its earliest stages the constituent cells much resemble those of the mesoderm by which it is surrounded ; that no other source for them was observed which appeared more probable ; and that in the embryonal development a portion of the dorsal nerve undergoes dissolution, its cells becoming transformed into the free mesoderm cells. It was conjectured that these latter might reassemble again to form the ganglion of the bud. But this view has, I believe, been given up by the author. The belief in a common mesodermal origin of hypophysis and ganglion, as described by Seeliger ('89), in the buds of *Pyrosoma*, appears to rest on a much securer foundation. Very recently Lefevre ('95) has maintained such an origin for the ganglion in *Perophora*.

In *Goodsiria*, the very early stage at which the ganglion is found to be fully separated from the hypophyseal duct, together with the conditions which I have observed in *Perophora*, have induced me to look for evidence of its mesodermal origin here. The close resemblance of its cells in its early stages to certain of the surrounding cells in the body space, *i.e.* mesenchyme cells, and the difficulty of observing its origin from any other source, are the only facts that lend any countenance to such a view — facts certainly of little weight, particularly when opposed to direct evidence of a contrary kind. What I shall have to say on this point when treating the gangleo-hypophyseal development in *Perophora* will be of greater moment.

The ectodermal origin of the ganglion in the bud has been asserted by Van Beneden et Julin ('87), for *Clavelina* ; by Oka ('92), for *Botryllus* ; by Salensky ('92), for *Pyrosoma* ; and by Brooks ('93), for *Salpa*. Of these authors' works the most important for us in the present connection is that of Oka. This is most important because of the obviously close relation between *Botryllus* and *Goodsiria* ; because of the positiveness of the author ; and because the work is very recent, and consequently was done by methods in common use at the present time, and in the light of prevailing theoretical views. It must, therefore, be examined critically. The author first describes and figures an irregular

accumulation of cells in the blood space between the already formed hypophyseal tube and the walls of the branchial and peribranchial sacs. This becomes more compact and regular in form; in short, it develops into the ganglion. Concerning the origin of the cells composing the mass, the author's essential words are: "Was die Herkunft dieser Zellen betrifft, so entstammen sie der Körperwand und zwar gehen sie durch Proliferation des dorsalen, oberhalb des hypophysären Rohres gelegenen Epithels hervor (Fig. 33). *Dieser Vorgang . . . kann nur in günstigen Fällen beobachtet werden*, denn die Zellen verlieren sehr rasch den Zusammenhang mit den Epithelzellen, von denen sie sich abspalten, und wandern einzeln oder gruppenweise rechts und links um das Rohr herum und sammeln sich an der unteren Seite desselben" (p. 540). The italics are mine, employed because these are to me the most significant words of the description. No one who has had experience in studying such a process as the author here so briefly describes (for the quotation contains all he has to say on the point) will be convinced by the evidence which he has produced that he has not fallen into error. Only a single figure illustrates his statements, and while in this two cells are shown which undoubtedly appear as though they might be in the act of leaving the ectoderm to enter the blood space, still in the absence of further illustration, or more precise statement, this instance cannot appeal to one as being necessarily more than a deceptive appearance.

The inadequate support produced by Oka for his view, together with the fact that he is opposed by all other observers (Della Valle, Hjort, and Pizon, who have studied the point in *Botryllus*), and likewise by my own results in *Goodsiria*, compel me to reject his conclusions.

Concerning the ectodermal origin of the ganglion in *Clavelina*, it is worth while to point out that Von Beneden et Julin have not given the subject special attention in their paper. Under the topic, "Development du coeur etc." they incidentally mention the ganglion several times, and it is shown in numerous figures; in most of them in close contact with the ectoderm, but also in *most quite distinct from it*. However, figures

representing sections of the ganglion well toward its posterior end show it apparently in organic connection with the ectoderm. Their most pronounced statement concerning its origin which I can find is the following, occurring on page 311: "Dans les dernières coupes il n'est pas possible de voir la limite entre l'ébauche neurale et l'épiderme ; elle paraît être un simple épaissement de l'épiblaste (Fig. 10, 11, et 12)." The authors' conclusions certainly need confirmation before they are entitled to unqualified acceptance.

The ectodermal origin of the ganglion asserted by Salensky and Brooks for *Pyrosoma* and *Salpa*, respectively, appears to be well supported in both cases, though it does not stand unchallenged, Seeliger ('89) claiming a mesodermal origin for it in both these genera. I have had no opportunity for personal observations on the point, and consequently shall express no opinion upon it further than this, that to my thinking even *Pyrosoma* is sufficiently remote in its relationship to the compound Ascidians to make possible an ectodermal origin of the ganglion in its ascidiozooids, while the same organ arises from the endoderm in the buds of the compound Ascidians. Seeing, as we do, the central nervous system arising from the *ectoderm* in the *embryozooids*, and from the "*endoderm*" in the *blastozooids* of the same species, its origin from either of these sources, or even from the mesoderm, in *Pyrosoma*, or still more in *Salpa*, ought not to cause great astonishment. That, however, there are such radical differences within the same genus as results indicate cannot be accepted till more evidence is at hand than we yet have.

The last-mentioned source of the ganglion, *i.e.* from the inner or "*endodermic*" vesicle, was asserted by several investigators whose observations were made a number of years ago, before the exacter methods of section-making had come into use in morphology, and before morphology had gone so greatly under bondage to the germ-layer theory as it has done more recently. And so it happened, as is not unfrequently the case in science, that the greater intellectual freedom enjoyed by the earlier workers more than offsets their cruder technique, and they were enabled to reach conclusions more nearly true

than are those reached by some later students under conditions reversed as to technique and intellectual freedom.

Among the earlier works to which reference is above made those of Giard ('72), and Kowalevsky ('74a and '74b), are particularly to be mentioned.

The results of my study of both *Goodsiria* and *Perophora* agree essentially with this last view; but since in neither genus were they reached without special perplexities, I must present the facts with considerable detail. In *Goodsiria* the formation of the ganglion is by no means a complicated process, but the difficulty in finding just how it occurs is due to the apparent quickness with which it becomes fully separated from its source, which is, I may say in a word, the ventral wall of the hypophyseal duct. At the time of writing my preliminary paper I was still in some doubt about its source, but more careful study since leaves, as I hope the following will show to the satisfaction of every reader, no room for question. Fig. 49, Pl. XVI, presents a transverse section of the duct in the earliest stage that I have found after its complete separation from the primitive inner vesicle. That the stage is one *very soon* after the separation takes place is certain from the state of development of the other organs of the bud as compared with the stage in which the duct is still merely a groove-like evagination. The peribranchial sacs are very slightly developed, the dorsal partitioning fold of the right side being barely indicated in this position, while the left does not appear at all in the section. Yet it is seen that the ganglion, *gl*, though very small, is already distinctly separated both from the wall of the primitive vesicle beneath it, and from the duct above it. The condition here shown, and even a slightly earlier one, but with the separation still complete, occurs not infrequently; but the critical stage, the one between this and that with the duct in the groove condition, escaped me for a long time, and made it seem quite probable that the cells entering into the ganglion were derived from some other source than the duct or primitive vesicle, *i.e.* either from the ectoderm or from the surrounding mesenchyme cells.

In only three or four buds have I found the decisive stages, but two of these, those from which Figs. 44-47, Pl. XV,

and Fig. 48, Pl. XVI, are drawn, are particularly to the point, and these I have consequently selected to describe. Figs. 45-47 represent transverse sections, in order from before backward, of a bud slightly less advanced than the one in nearly longitudinal section shown in Fig. 48. The sections are considerably oblique, so that the one, Fig. 44, passing through the mouth of the duct makes it appear to be inclined to one side. The ganglion is not touched by this section, my purpose in figuring it being to show that the hypophysis mouth exists at this time, though very small. This section, with the following one, not reproduced, leaves no doubt on the point. Figs. 45 and 46 are from sections a little farther back. They need very little explanation. They show the thickened ventral wall of the duct, which is (particularly in Fig. 46, six sections farther back toward the place where the duct is still unsevered from the vesicle) not distinguishably separated from the wall of the vesicle. In this thickened region, Fig. 46, cell division is more frequently seen than in other parts of the tissues. Fig. 47, one section farther back, shows that the duct still communicates by a wide opening with the primitive vesicle. Three more sections take us beyond the duct entirely, so it is obvious that the communication shown in Fig. 47 is at the posterior part of the duct. If now we turn to the longitudinal sections, one of which is shown in Fig. 48, Pl. XVI, we find that the lumen of the duct no longer communicates with the vesicle posteriorly, but that there exists a trace, *v.c.o.*, of the opening by which this communication was earlier effected. But the most important fact to be learned from this section is that anteriorly the ganglion, *gl.*, has now become separated both from the ventral wall of the duct and from the wall of the vesicle; but that posteriorly, where the duct has not yet fully severed its connection with the vesicle, the ganglion is still confluent with the large cell mass that forms at the same time the ventral wall of the duct and the dorsal wall of the vesicle.

We are thus led to recognize that *simultaneously with the closing off from the inner vesicle from before backward of the hypophyseal duct, the ganglion becomes differentiated in the same*

order from the cell mass that forms the last connection between the duct and the vesicle.

e. The Genital System.

The results obtained from the study on this system are far from complete. It is very imperfectly developed in all my specimens — so much so, in fact, that I cannot regard as final any of the observations made. As already said, my material was all procured in one locality, and at the same time, *viz.*, December 30 — midwinter. It is not at all impossible that when opportunity is afforded to examine specimens taken from other places, and particularly at other seasons of the year, the sexual organs will be found to tell a different story from that intimated by the fragmentary facts presented by the colonies so far studied.

The character of the sexual organs in the adult, already described, and the manner of origin of the sexual cells in the *Botryllus* bud, lead us to expect that here also we shall find the youngest ova and sperm cells floating free in the body space, having been brought hither by the blood directly from the parent zooid. That such is the case seems quite certain from the facts observed. In several instances I have found ova in the blood (Fig. 52, Pl. XVI), but it is noteworthy that in all these cases they were in *buds far advanced in development*. Although I have given particular attention to the point I *have not yet succeeded in finding an undoubted case of sexual cells in a very young bud*. This seems the more remarkable when it is remembered that the bud becomes fully severed from the parent at a very early stage in its development. But the young buds examined are far too few to justify the negative conclusion that recognizable ova never enter them before their complete severance from the mother zooids. Nevertheless the facts observed are of a kind and sufficiently numerous to make very interesting the several alternative questions which they raise. Direct observation shows that in some colonies in which some of the blastozooids contain ova and are consequently presumably capable of sexual reproduction, certain other zooids are developed without having received recognizable ova directly

from their parents. Do these latter zooids remain throughout their lives incapable of sexual reproduction; or do they receive their ova from the blood vessels with which, as previously shown, the zooids become secondarily connected; or have some of the cells contained in the blood the capability of being transformed into sexual cells; or finally, are the sexual cells really brought into the bud from the parent, which again received them in the same way from its parent, and so on till their ultimate origin was the sexually produced common ancestor of the entire colony? The last is, I suppose, the alternative that would appear most probable to the great majority of embryologists, since it is the one most in conformity with prevailing theoretical views concerning the origin of the reproductive elements. It is also in keeping with the early appearance of the sex cells in the *Salpa* stolon, as made known by Kowalensky, Salensky, Brooks, Seeliger, and others; also with what occurs in the budding of *Pyrosoma*.

But most of all it is supported by the conditions presented by the buds of *Botryllus*. The large ova in the young buds of this species, known since the time of Savigny ('16), have by many writers been supposed not to appear in any of the buds before the third or fourth generation. Pizon ('93), has, however, shown that they really originate in the sexually produced embryo, and migrate, as he expresses it, through the first generations of buds, leaving these to develop into sterile zooids, only to become permanently fixed as ovaries in later generations.

The fact that I have not been able to distinguish sexual cells among the blood cells in the young buds of *Goodsiria* may not be regarded as proof that they do not exist there. They may be in so early a stage of differentiation that, with the methods of preservation and staining employed, their distinguishing characters were not brought out, as they might have been by some other treatment.

It is true that I have occasionally found large polynuclear cells (Fig. 55, Pl. XVI) that have seemed to me to be possibly the forerunner of the cell aggregates. These I have again conjectured to be a stage in the formation of the polycarps.

But I have been unable to get decisive evidence of such a relation between these elements; and the structure of the youngest undoubted female polycarps found does not strengthen the conjecture, for in these the ova are in different stages of development, but even the smallest show the characteristics of ova. Fig. 51, Pl. XV, represents such a polycarp. It has not yet advanced sufficiently to have pushed its way from the body space into the peribranchial chamber. Certain clusters of a few cells larger, more deeply stained, and more granular than the other blood cells, Fig. 54, Pl. XVI, I suppose to be the male sexual cells; but this too is not beyond question.

As has been pointed out in describing the species, the polycarps have a rather regular position on the ventral side of the zooids, on each side of the endostyle and not far from it. In other words, they occupy undoubted determinate positions. With the sex cells at first apparently wholly subject to the movements of the blood, one would much like to know how it comes about that their final points of attachment, where they develop into the polycarps, are always nearly the same. That they are in the ventral part of the zooids suggests that the greater weight of the cells, while still swimming in the blood, prevents their being carried into the higher parts to become attached; but that indicates nothing as to why they should become arranged so regularly on each side of the endostyle. Unless we may assume determinants for these particular cells of a considerably higher grade of intelligence than is generally attributed to these convenient phantoms, it would seem that there must be some physical or chemical condition in the membrane in the region where the polycarps are situated that attracts to it the sex cells from the blood when they happen to be carried near it.

With the young sex cells entirely subject to the movements of the blood, and as a consequence liable to be carried to various parts of the colony, — into one zooid or another, — it is interesting to notice how completely, from the sexual point of view, at least, the *colony*, and not the *ascidiozooids composing it*, is the individual.

B. PEROPHORA ANNECTENS, RITTER.

I. INTRODUCTORY. MATERIAL. TECHNIQUE.

I have described this species in detail in a former paper ('94), and consequently am relieved from doing so here. One point only concerning it I must speak of briefly. In the description referred to, I have said in substance that the species includes at one extreme colonies in which the zooids are quite as distinct from one another as they are in *P. Listeri*, for example, they being nearer together, merely, on the stolons; and at the other extreme colonies in which the zooids "are as completely enveloped by a common test as they are in *Botryllus* or *Goodsiria*."

I wish here to reaffirm and emphasize this statement in its fullness. I have several times reexamined my material with a view to finding some constant structural difference that would enable me to separate specifically the one extreme from the other; but each effort has resulted in strengthening the conviction that such a separation is impossible. It may be worth while to add that I have reexamined the case since having had opportunity to study several other species of *Perophora*, new and old, *P. Listeri* included. Last summer I had the pleasure of submitting my specimens to the experienced judgment of Prof. Herdman, and he agrees with me fully that they are all one species. I reassert, then, *that we have here within the limits of the variability of a single species a complete transition from the Simple or Social Ascidians (depending on what author's system of classification be adopted) to the Compound Ascidians*; or in other words, a complete transition within a single species between two groups that have been considered as distinct families, Bronn, Gegenbaur; or as distinct suborders, Herdman; or even as distinct orders, Haeckel, Claus. For a summary of the various systems of classification that have been proposed from time to time, see Seeliger ('94). Lahille ('90) has proposed a wholly new classification and nomenclature of the groups above genera, in which the simple or compound conditions are entirely ignored as differentiating characters for

any groups higher than species. To some extent this radical departure receives support from *Perophora annectens*. But I fully agree with Herdman and Seeliger in thinking that the system adopted by Lahille, at any rate as regards his families and suborders, comes no nearer being a natural one than the other systems which it is intended to supplant. But it would be outside the purpose of the present paper to enter upon a general discussion of tunicate classification.

In view of the apparent hesitation of some students of the Tunicata, with whom I have had personal conversations, to accept my conclusion that *P. annectens* really does include such widely varying forms, I had thought to present in the present publication a more detailed and fully illustrated description of all the transitional forms than is contained in my former paper. However, further reflection has made it seem to me that such a discussion will belong more appropriately to a comprehensive treatment of all our Pacific Coast species of the genus. Such a treatment will be contained in a monograph of the California Tunicata which is now in course of preparation by myself and one of my students, Mr. F. W. Bancroft.

As stated in my preliminary, my results on the budding of *Perophora* have nearly all been obtained from the study of *P. annectens*. From the abundance of this species in Monterey Bay, Cal., and from the compactness of the colonies, it is very easy to get unlimited material, and whenever I have collected it, the buds in all stages of development have been present in great numbers. *P. Listeri*, on the other hand, at least in the Bay of Naples, I found to present almost insuperable difficulties in the way of procuring buds in sufficient quantities to enable me to get the necessary stages, and those in such numbers as to make a satisfactory study of their development. Both the zooids and the stolons are very delicate, and they cling so closely to the stones on which alone I found them that the young buds are removed with very great difficulty. I succeeded, however, in securing specimens enough to enable me to make sure that there is no difference of moment between the bud-development in the two species.

For the killing and preservation of material I have used the various methods that have been recommended by the numerous investigators who have been occupied in recent years with Ascidian morphology and development, but in spite of the variety of treatment I have not been able to get preparations of this genus quite as satisfactory as those obtained from *Goodsiria*. On the whole, as in *Goodsiria*, the specimens fixed by the weaker solution of the picro-sulphuric mixture of Kleinenberg has given the best results; but the glacial acetic acid method and the chromic-acetic acid solution have also been found useful.

Several zoologists have studied the budding of *Perophora*. Giard ('72), Kowalevsky ('74), Van Beneden et Julin ('87), and Pizon ('93), have published their observations on the subject, but of these by far the most important work is that of Kowalevsky. It was this investigator who first described in detail the entire development of the blastozoids, and his results were so nearly complete that the points which he left in doubt, with perhaps a single exception, remained in that condition up to the present time, notwithstanding the studies that have since been bestowed upon the subject. The points to which I refer are the origin of the ganglion, the heart, the sexual organs, and the precise relation of the zooids to the stolon; or more exactly, to the cloison, or septum of the stolon. The first, second, and fourth of these, as the sequel will show, have been rather obscured than clarified by the more recent works. Concerning the third it appears that the results of Van Beneden et Julin are more exact than those of Kowalevsky; but of this I am unable to judge from personal observations. In none of the specimens studied by me have the genital organs been sufficiently developed to permit a satisfactory study of them.

Because of Kowalevsky's paper a redescription of the general development of the buds would be quite superfluous. Excepting the genital system, I shall confine myself, therefore, to the points mentioned above as having been left doubtful by him.

2. RELATION OF THE BUD TO THE STOLON.

The point to be made in this connection may be briefly stated by quoting what I have already said in my preliminary paper: "When the differentiation of the 'endoderm' into the branchial and two peribranchial sacs takes place, it does so in such a way that the developing blastozoid is connected with the double-walled partition of the stolon, not by the *branchial sac*, as has been hitherto supposed, but by the left *peribranchial sac*."

Figs. 57-64, and 65, 67, and 70, Pl. XVI, will suffice to illustrate the facts. Of these Figs. 67 and 68 are from the youngest stage. At this time the inner vesicle is still almost a perfect sphere, no differentiation of organs having yet begun excepting that a mere trace, *pc.a.*, of the heart is present. The connection of the inner vesicle to the stolon, *cl'n.*, does not show in his section, but it does in the third section of the series preceding this. The bud at this time does not stand out at a right angle to the stolon, but is inclined somewhat toward its tip. The series passes from the posterior, or point of attachment of the bud, toward its anterior, *i.e.* toward the tip of the stolon. It is for this reason that we find the section of the stolon, septum in several sections before we come to its place of attachment to the inner vesicle.

The process by which the vesicle becomes divided up into the branchial and two peribranchial chambers differs so little from the same process in *Goodsiria* and various other Ascidian buds, that I have thought it would be sufficient to pass over the various stages excepting those that pertain especially to the condition about to be described. It is, however, necessary to refer to the account given by Lefevre of the first stages in the differentiation of the vesicle. He states that in *P. viridis* the inner vesicle rotates to the right through 90° during the initial steps in the formation of the peribranchial sacs. According to him the heart *Anlage*, the first organ to appear, is located at the beginning 90° farther to the dorsal and left of the vesicle than the position that it ultimately occupies in the

adult zooid. The rotation is not so great in *P. annectens*, as may be determined by considering Fig. 67. The heart *Anlage* is certainly somewhat higher than it will be at a later time, as its position with reference to the stolonial septum, in this section shows. I have seen no specimens in which the *Anlage* was at a higher point, and I think that this particular individual furnishes evidence that no appreciable rotation could have taken place in the earlier stages of development. This evidence consists in the uniform thickness of the wall of the vesicle in all its parts, thus indicating that there has been no considerable inequality of growth, to which Lefevre probably rightly attributes the rotation; and to the fact that at this time the connection of the septum to the vesicle is not yet thrust off to one side as it later comes to be (Fig. 70) when the rotation is complete. It is undoubtedly to this rotation that the attachment of the septum to the left peribranchial sac is due.

The greater or less extent of the rotation in various species probably has no particular significance beyond the general significance that attaches to variations in development.

From this early stage we may now pass to the examination of one much farther advanced. Such a one is shown by the series of Figs. 57-64, all from the same bud. The sections pass from the anterior toward the posterior of the animal, and are seen on their anterior surfaces, thus causing an apparent reversal of right and left. In Fig. 57 the peribranchial sacs are entirely distinct from the branchial sacs, and this is the condition for a considerably greater portion of the anterior part of the bud. Fig. 58 shows the point at which the three cavities become confluent. From here the ventral partitioning folds, *v.f.*, rapidly fall away; compare Fig. 59, seven sections farther back. A few sections farther back (Fig. 60) they have almost wholly disappeared, and the three cavities have become practically one. This section, with those going before it, shows clearly, however, that the bay, *l.pb.s.*, is the posterior tip of the left peribranchial sac. But this is the point at which attachment to the stolonial septum, *cl'n.*, occurs. It will be seen that the ventral side of what will later be the branchial sac is far below this, it being marked by the already forming endostyle, *end.*

Series of figures similar to these, representing both younger and older stages, might be multiplied indefinitely were it necessary; but the main facts, *viz.*, those concerning the relation of the zooid to the stolon septum, are so clear that no further illustration is required. And this is the more so because Lefevre (95) fully confirms my results in this particular. In Fig. 65 I have shown a single instance from many that might be given of the same relations in a bud of *Perophora Listeri*.

The connection between the septum and the peribranchial sac becomes entirely lost at a stage only a little later than that represented in the series 57-64. Lefevre states that the severance does not occur until a somewhat later time in *P. viridis*. This difference in the two species may be correlated with the fact that the zooids of the *P. annectens* colony are closely bound together by the test, and hence depend less on the stolon for maintaining their colonial character than do those of *P. viridis*. It is certain that in some cases, at least in *P. annectens*, the buds become wholly severed from the stolon, the ectodermal as well as the endodermal connection being cut away. I have shown an instance of this in Fig. 66. This is the section of a complete series in which the stolon, *sto.*, with its septum approaches most nearly to the wall of the zooid; yet there is a distinct absence of connection between them. I am unable to say with what frequency this complete separation takes place, but so far as I have been able to determine it appears that it is rather exceptional. Certain it is that the stolons of a colony are always in communication with many of the zooids because the blood is always in motion in the living colonies, and there is, of course, no other propelling power than the hearts of the zooids.

I have stated that the connection is always to the *left peribranchial sac*. This has been true for every undoubted instance observed, but it does not seem in the least improbable that exceptions might be found were one to examine a sufficiently large number of cases; though that it is well-nigh the invariable rule is certain from the large number of instances of its occurrence that have been observed.

That Kowalevsky failed to make out the precise relations between the bud and the septum of the stolon is not surprising, since he studied whole buds almost exclusively; at least he could have had no complete series of sections. The septum is very delicate, and is entirely surrounded by tissues denser than itself, and for these reasons it is almost, if not wholly, impossible to trace the actual condition of things in the living bud. But the failure of Van Beneden et Julin, and of Pizon, I cannot account for so easily, since these authors made use of thin sections in their studies. It seems probable that they either missed the stages in which the facts are most easily seen, or that their series of sections were imperfect. Under other circumstances it appears hardly possible that they would have overlooked the facts, patent as they are.

It would appear that Van Beneden et Julin, having carefully followed through its entire development the bud of *Clavelina*, and having studied the bud of *Perophora* sufficiently to see that in most particulars it agreed with *Clavelina*, thought themselves safe in assuming the same agreement would hold in all points. So many common characters do the two genera present, not only in adult structure but also in development, both of embryozoids and blastozoids, that such an assumption might very naturally seem warranted. The case furnishes only another illustration of the dangers to which morphologists are subject when they assume certain things to be true of one animal because they are known to be so for another closely related one. The fatality lies most frequently, I suspect, in the supposed, and not real, close relationship between the animals.

These authors practically, though unconsciously, say that their observations on this particular point are defective for *Perophora*. They say (p. 307), "nous n'avons pas pu nous édifier complètement sur l'histoire du coeur et du système nerveux," etc. Now, as will appear from my account of the development of these organs, it would have been well-nigh impossible for them to fail of a clear understanding of the development and relations of structures mentioned without likewise missing the true relations of the bud to the stolonian septum.

That they did suppose the same conditions to hold for both genera is evident, although they say so rather indirectly. Thus, in summing up their results on the development of the heart "et de ses dependances chez la Claveline," they say, p. 317, "Chez la Claveline, comme chez la Pérophore, la vesicule interne du burgeon," etc. ; and then follows a brief restatement of the relations between the primitive inner vesicle, the branchial and peribranchial sacs, the epicardium, the pericardium and heart, and the cloison, as they have been described in detail for *Clavelina*. In this connection I shall merely say that the description which they give for *Clavelina* does not apply in a single essential particular, excepting as to the formation of the heart from the pericardium, to what actually takes place in *Perophora*. In describing the development of the heart I shall point out in detail the difference between the two.

Pizon's paper is entitled "Histoire de la blastogénèse chez les Botryllides," and the author does not claim to have made so exhaustive a study of the numerous other genera which received his attention as he has of *Botryllus* and *Botrylloids*. As regards *Perophora* he seems to have examined it just enough to make himself feel safe in adopting the errors on this point, at least, into which Van Beneden et Julin had fallen. Thus, he says, p. 105, "Disons d'abord que chez des bourgeons d'*Amaroncium proliferum*, de *Circinalium concrescens* (Giard), de *Perophora Listeri*, de *Clavelina Rissoane*, je suis arrivé identiquement aux mêmes résultats que Kowalevsky (*Am. proliferum*) et que Van Beneden et Julin (*Clavelina Rissoana*) à propos de l'origine du tube epicardique." Then follows a brief description of the epicardium and its relation to the branchial sac and pericardium, which is the same as that already referred to as having been given by Van Beneden et Julin. But this writer's statements on this point I shall also have to consider further after having described the development of the heart.

3. PERICARDIUM AND HEART.

It appears to be the rule that the pericardium is the very first organ to be founded in the *Perophora* bud. At least, I have found a structure present in several buds at a time when the primitive inner vesicle is still wholly unmodified, which I suppose to be the *Anlage* of this organ. One of these is represented in Figs. 67 and 68, Pl. XVI.

My reason for thinking this to be the beginning of the pericardium is this: the sections of this bud are cut from the posterior toward the anterior end of the future zooid, consequently they are seen in their natural position as regards right and left. This, as I have pointed out a few pages earlier, makes this structure in the position, the slight lateral rotation of the vesicle to the right having been performed, that the heart occupies in the adult bud. The only question that could be raised against this identification would rest on the possibility, first, of error concerning the anterior and posterior ends of the bud; and second, that in this instance we have an exception to the rule that the septum is connected with the left peribranchial sac. As regards the first, the possibility of error is remote because another older bud in the immediate vicinity is clearly cut from behind forward, and the two are so close together that a reversal of their anterior ends is hardly possible. As regards the second, nothing can be said beyond what was asserted in discussing the relation of the bud to the stolon; *viz.*, that in the large number of buds observed, no exception to the rule has been found. If this is an exception, it is the only one hit upon.

We may then regard it as certain that the cell mass under consideration is the *Anlage* of the pericardium. The origin of it is a difficult matter to decide. The numerous and widely separated groups of Tunicates in which the pericardium has been satisfactorily shown to arise from the endoderm is a circumstance that in itself makes its similar origin here probable; and my observations on the whole point in the same direction, though I have been unable to remove from my mind some traces of doubt on the subject. As shown in Fig. 68,

there seems to be in the section here represented a passing of cells from the wall of the vesicle into the *Anlage*, *pc.a.*, but the evidence of such a process is more convincing in this section than in either of the other three sections of the series in which the *Anlage* appears ; and even here, as the figure shows, the line of separation between the wall and *Anlage* is distinct in places.¹ At the same time the cells of the *Anlage* are somewhat more deeply stained than are the immediately adjacent ones in the wall of the vesicle, a fact which I have attempted to bring out in the figure by making the former slightly darker than the latter. Again, as seen by this figure, the cells of the *Anlage* are not closely massed together as one would suppose they would be had they been derived from the vesicle at the single point where, as shown by the figure, they are passing from the latter into the former.

These two last-mentioned conditions raise some doubt about the origin of the *Anlage* from the vesicle, and at the same time they, together with the close resemblance of the cells composing it to some of the surrounding mesenchyme cells, suggest these latter as being its source. I have not seen an earlier stage than this in the formation of the *Anlage*, but have found about the same and slightly older stages not infrequently, and in all cases the conditions presented are very similar. In Figs. 69 and 71, Pl. XVI, are represented sections from different buds both considerably more advanced in development than that just described, though of the two the one shown by Fig. 71 is somewhat older. Here the cavity of the pericardium is already established, though it is quite small, particularly in the younger bud. The portion of the wall of the organ in contact with the wall of the inner vesicle is thicker than elsewhere, and the two appear to be in organic connection ; but any one who has had experience in determining whether two cell masses in contact with each other are really organically connected or not, knows well how extremely easy it is to be deceived. I have carefully examined with an oil-immer-

¹ The interruption of the line separating the *Anlage* from the wall is at a point toward which the index line *pc.a.*, Fig. 68, points. The lithographer has failed to accurately reproduce my drawing here.

sion lens all the sections of these two, and numerous other cases, and believe that the connection is real and not apparent; and since at a later stage the separation certainly becomes complete, the connection cannot be supposed to be secondary. I therefore conclude *that the pericardium originates from the wall of the primitive inner vesicle.*

At the same time, however, I must expressly state what is already obvious from the description, that the conclusion rests upon a *preponderance of evidence*; there is certainly some evidence against it, and that is indicative of a mesenchymal origin of the cells.

(Since the above account of the origin of the heart was written, Lefevre's short paper has reached me, in which he affirms that the mesenchyme is the source of the heart. I have reexamined my preparations with much care, and must say that, although, as my words above indicate, Lefevre's statements found my mind in a condition of equilibrium, almost, on the point, I am still of the opinion that my conclusion is correct, at least for *P. annectens*. In fact, my more recent study has discovered some additional evidence in support of my earlier conclusion. For example, Fig. 69 represents a section in which, *at the focus here shown*, as seen under the oil-immersion lens, it is certain that no interrupting line is present, and at β is one cell, at least, that is undoubtedly about to divide, though I do not stake much on this fact. A circumstance connected with another section of this same series is I think quite indicative that the *Anlage* is in organic connection with the vesicle. It is this. The outer thin wall of the pericardial vesicle has been, in the section referred to, by some means artificially broken away from the rest of the vesicle; yet the thickened side next the endodermic vesicle is still as indistinguishably confluent with the latter as in the section figured.

It seems to me quite likely that a force considerable enough to break this wall would have moved the whole pericardial vesicle from its contact with the endoderm were its relation merely a contact.

I have found no sections in which *at some focuses* I cannot see the separating line to be uninterrupted; but, very distinctly

in many sections, and less distinctly in many others, I am sure that the line is interrupted.

And, as already said, the fusion can hardly be regarded as secondary, because at a little later time the pericardium is clearly wholly distinct from the "endoderm." Now what is the explanation of the facts that have left traces of doubt in my mind, and have led Lefevre to believe that the cells under consideration come from the mesenchyme? I believe it to be this: *That the mesenchyme cells themselves are being constantly produced from various parts of the endodermic vesicle for a considerable time during the early stages in the development of the bud.*

My evidence for this is not as conclusive as we would wish it to be, but at the same time I have observed a considerable number of cases in which I believe cells are being set loose from the "endoderm," and are passing into the blood. Figs. 77 and 78, Pl. XVII, represent cases of this kind, *b.c.*¹, being the cells referred to. These are both remote from the position at which either the ganglion or heart will form.

And, on theoretical grounds, is not such an origin of the mesenchyme cells highly probable? Certainly new ones must be forming rapidly somewhere, for the newly developing zooids must make large demands on them for their blood and muscles, both of which undoubtedly come immediately from the mesenchyme. To suppose that all the muscle, blood, and mesenchyme cells of a colony have been derived directly from the mesenchyme cells of the embryozoid does, it seems to me, rather more violence to general developmental principles than to suppose that each new bud is capable of producing such cells for itself. And what part of the bud is more likely to do this than the "endoderm"? If my belief on this point is correct, then it is not at all impossible that the heart, or even the ganglion, may be formed, at least in part, from mesenchyme cells, for the mesenchyme cells would themselves have the same source, and when first severed from the "endoderm" would be of the same character as the cells which certainly produce the heart and ganglion in the buds of some other species. It would be merely a question of what position on the primitive inner vesicle the cells should be given off. And

in this connection it is significant that the resemblance of the *Anlage* cells to the mesenchyme cells does not apply to *all* the mesenchyme, or blood cells by any means. As a matter of fact, it is to a comparatively small number of these latter that the former have such a resemblance.)

A detailed account of the development of the heart from the pericardial vesicle is just as little necessary here as in the case of *Goodsiria*, where it was stated that such a description would be superfluous, so entirely does the process correspond with what has been many times described for other Tunicates. Fig. 61, *ht.*, Pl. XVI, incidentally shows the heart beginning to form by an invagination of the large pericardial vesicle.

The matter of chief interest in connection with the development of the heart in the buds of *Perophora* remains to be considered. I refer to its *direct* origin from the primitive inner vesicle, and its consequent independence of the stolonial septum. The facts in relation to the subject were partly presented, as will be recognized, in describing the manner of attachment of the blastozoid to the septum; and the whole question might have been very properly discussed there. But, since it has been more commonly treated in connection with the heart by other authors, it seemed best to adopt the same plan in this instance. It has already been pointed out that Van Beneden et Julin supposed *Clavelina* and *Perophora* to agree in this respect, as they do in many others. In order to make it clear that they do not, it will be necessary to point out what, according to these authors, are the conditions in *Clavelina*. An understanding of them in all their details can hardly be reached, however, without aid of the numerous figures by which the authors have illustrated the subject. These are all the figures of plate XI; figures 1 to 7, plate XV; and figures 3^a, 3^b, 3^c, 3^d, and 3^e of plate XVI of their well-known memoir ('87).

The parts essential to an adequate understanding of the conditions described by them are: the primitive inner vesicle, the branchial sac, the "tube épocardique," the "sac épocardique," and the "cloison stoloniale."

To make the subject clear, and at the same time to do so as

briefly as possible, I will quote their words in part, and in part give my own description condensed from theirs. On page 317, previously quoted in part, they say "Chez le Claveline, comme chez la Pérophore, la vésicule interne du bourgeon résulte de l'écartement des deux lames cellulaires adjacentes de la cloison stoloniale. La vésicule, allongée dans le sens de l'axe du bourgeon se continue en arrière dans la cavité virtuelle de la cloison stoloniale. *Cette vésicule se divise transversalement en une portion terminale et une portion basilaire. La portion terminale de la vésicule donne naissance au sac branchial et au tube épicaudique. . . . La portion basilaire engendre le sac péricardique. . . . Puis, par une sorte d'étranglement progressif, les deux portions de la vésicule interne primitive se séparent l'une de l'autre.*" The italics are mine, and are inserted to call attention to the points most important for the present purpose. One might understand from the first part of the quotation that the "portion terminale" is fully separated from the "portion basilaire" before any farther differentiation; but such is evidently not the meaning, since the lines omitted between the last and next to the last parts of the quotation give the character of the connection between them and the relation of the whole to the developing heart. I should perhaps have included in the first part of the quotation another line or two which state that the "portion terminale" gives rise also to the peribranchial sacs and the intestine.

On pages 315, 316 the following is much to the point. "Il résulte de l'examen de la série des coupes successives faites à travers le bourgeon partiellement représenté pl. XVI, figure 3^a à 3^e, que le *péricarde*, l'*épicaudique*, et le *sac branchial* sont des parties, incomplètement séparées l'une de l'autre de la vésicule interne du bourgeon. . . . Cependant, le sac péricardique a commencé à se séparer de l'épicaudique, et l'étude de ce bourgeon nous permet de nous faire une idée très exacte de la manière dont s'accomplit cette séparation." Then follows a description of the way in which the separation takes place, and still further on the statement: "Plus tard ces communications cessent d'exister et dès lors la vésicule interne primitive du bourgeon s'est subdivisée en deux parties distinctes: *sac branchial* et

épicarde, en avant *péricarde* et *cloison stoloniale* en arrière." And on another page they show that the cavity of the *pericardium* is directly continuous into the virtual lumen of the *cloison*, and that this is the condition retained in the adult zooids.

The essential facts here set forth, summed up in the fewest words possible, are these: The epicardium is derived from the branchial sac, or more precisely from the part of the primitive inner vesicle that later forms the branchial sac. The pericardium is derived from the epicardium. The epicardium remains in connection with the branchial sac, but becomes fully separated from the pericardium. The pericardium remains in connection with the *cloison*.

That the epicardium is a well-defined structure in *Clavelina*, is obvious from the description and figures of it by the authors. Thus it communicates with the branchial sac by two openings called by them "*orifice épocardique*," these being in reality two short tubes passing between the branchial sac and the "*tube épocardique*" proper, which is a single wide cavity terminating posteriorly in two "*cul-de-sacs épocardiques*."

That the course of things in *Perophora* is very different from this is clearly seen by an examination of the series of Figs. 60, 61, and 62, Pl. XVI. The most striking difference is in the fact that in *Perophora* the pericardium *neither at its origin, nor at any later time, has any connection whatever with the cloison of the stolon*; see particularly Fig. 70, Pl. XVI, from another somewhat younger bud. From these it is seen that it arises directly from the primitive inner vesicle on its *right* side, consequently remote from the point of attachment of the *cloison* to the inner vesicle, which point is on its *left* side, corresponding in this stage of development to the posterior extremity of the left peribranchial sac. This difference implies the further one *that there is no epicardium in Perophora*, at any rate holding such a relation to the pericardium as this structure does in *Clavelina*. It may be asked if the portion of the primitive vesicle from which the pericardium is derived may not be regarded as representing the epicardium of *Clavelina*. It certainly does hold the same relation to the inner vesicle; *i.e.*

it is at its posterior middle part, so that later it becomes a posteriorly extended portion of the branchial sac; compare Figs. 62, 63, and 64, in which *br.s'* indicates the portion of the vesicle referred to. But it must be noticed that this is nothing more than the somewhat narrowed rear end of the branchial sac leading to the oesophagus (*oe.*, Fig. 62), and extends back only a very short distance behind its opening. Fig. 64 represents a section only three sections, $7\frac{1}{2}\mu$ thick, farther back than the one shown in Fig. 62, passing through the opening of the oesophagus; and in this it will be noticed that the cavity *br.s'* does not appear, though the stomach does. This is practically the condition found in the adult zooids. However, the fact that this coecum, as it may be called, is the vestibule to the oesophagus need not, in view of its relations to the branchial sac and pericardium, stand seriously in the way of regarding it as representing the epicardium. But to make it correspond with this structure in *Clavelina*, its relation to the septum would require such a radical transformation of things as makes it very difficult to believe that the two structures are genetically related to each other. To shift the connection of the septum from the *peri-branchial sac* on the *left side* of the body, to the *pericardium* on the *right side*, would be a rather serious matter, it seems to me.

Having thus shown a radical difference between *Clavelina* and *Perophora* in the relations of the pericardium to the stolonial septum, and of the septum to the branchial apparatus, I leave the subject for the present to resume it again later on from a more general point of view.

4. THE HYPOPHYSIS AND GANGLION.

It was pointed out in my preliminary communication that while in both *Goodsiria* and *Perophora* the investigation of this subject encounters special difficulties, it fortunately happens that the difficulty is not at the same point in the two genera.

In *Goodsiria*, as we have seen, the origin of the *common Anlage* of duct and ganglion from the *inner vesicle* is observed

with the greatest ease and certainty; the difficulty is found in ascertaining the *source of the ganglion*. On the other hand, as we shall presently see, in *Perophora* the origin of the *ganglion from the common Anlage* is seen with perfect distinctness, while the *source of this common Anlage* is ascertained with considerable difficulty.

It is a suggestive fact that in *Perophora* the difficulty encountered in making out the origin of the ganglio-hypophyseal *Anlage* is precisely the same as that which we have already seen in the way of ascertaining the source of the pericardium. In *Goodsiria* it will be remembered that we found both hypophyseal duct and pericardium to arise as well-defined evaginations from the primitive vesicle. In *Perophora*, on the contrary, we have already seen the pericardium arise, almost certainly from the inner vesicle, but, instead of by an evagination, by a cell egression so difficult to observe that a trace of doubt might be entertained as to whether the cells have in reality come from the vesicle or from the surrounding blood, or mesenchyme cells. We shall now see the ganglio-hypophyseal *Anlage* to arise in the same way as the pericardium, and hence in the same way its source is not as absolutely beyond question as might be desired. In both genera it appears as though the influences which have determined the character of the development of one of these structures have also determined that of the other.

Since in *Perophora* the cloudy point is not the origin of the ganglion alone, as in *Goodsiria*, but of the common *Anlage* of duct and ganglion, my reference to the four different alleged sources of the ganglion, made when treating the development of that organ in the latter species, should be recalled here and considered in connection with the origin of the ganglio-hypophyseal *Anlage*.

The supposition that it originates directly from the nerve ganglion of the parent zooid would, in this instance, be so obviously unjustifiable that Pizon himself would hardly venture to make it.

In my preliminary I have declared it to be "absolutely certain" that the *Anlage* does not arise from the ectoderm.

This declaration was made in the face of a full appreciation of the general danger there is in unqualified affirmations that certain developmental processes do *not* take place ; but in this instance I believe such positiveness is justified. I must give my reasons for believing so with particular care and detail, because of the slight uncertainty of my conclusion that the inner vesicle is the source of the *Anlage*, and furthermore because of the supposition by Van Beneden et Julin ('87) that the ganglion originates from the ectoderm in the *Clavelina* bud.

It is in the *character of the ectoderm* and the *relation of the Anlage to it* that my conviction finds its justification. The ectoderm is composed of a single layer of cuboid cells, so large, regularly placed, and distinctly set off from one another that they are quite diagrammatic in their clearness in most preparations. The nuclei are round, and generally sharply contrasted with the cell-body by their distinct membrane and their less deeply stained ground substance. They are as a rule situated somewhat nearer the inner side of the cells.

The inner surfaces of the cells are remarkably even and clear cut, and the layer of protoplasm forming them appears to be denser than the rest of the cell-body ; at least, it is generally stained more deeply (Figs. 68, Pl. XVI, and 72 and 74, Pl. XVII). From this character of the individual cells the inner surface of the layer which they compose appears in sections as a very sharp line ; and as this line would have to be broken were cells to enter the body space from the layer, either by cell division, or by migration, I cannot believe the process could escape all the search I have devoted to the point. Further than this the distinctness of the cells from those adjacent to them in the body space and those composing the *Anlage* is evidence to the same end. Their nuclei are in general smaller ; but the most important difference is in their behavior toward reagents. By some methods of treatment, most markedly apparent perhaps in some specimens preserved in Perenyi's fluid and stained with Kleinenberg's Haematoxylin, the protoplasm of the ectoderm cells, after having been decolorized, shows a dark dirty greenish tint that is entirely characteristic of them, not only

as compared with the blood and *Anlage* cells, but also as compared with any other cells whatever of the animal.

And with almost all the methods of preparation used the ectoderm cells stain considerably more deeply, *particularly on their inner sides*, than do the other cells with which we are concerned. In some instances where the blood cells are particularly numerous between the ectoderm and the inner vesicle, it is wholly impossible to decide whether the "mesenchyme" cells are being given off from the inner vesicle or not, so much do they resemble the cells of the latter, and so imperfect is the separating line between them. But in these cases there is *not the least possibility of deception about the distinctness of the body-space cells from the ectoderm*. The difference in staining and the clear boundary line, as described above, preclude it. Now of course, a critical reader, particularly if he be inclined to be skeptical, might reply that for a particular instance it may be true that the case against the ectoderm is as clear as here presented, and that in this instance no cells are being given off from it; but that this is very far from proving *that at some other stage in development, or in some other region* this process does not take place. I fully appreciate the weight of this rejoinder, but in this instance think it wholly over-balanced. As to the second part of it, I would say that the descriptions and figures all apply to the ectoderm on the dorsal side of the bud immediately over the *Anlage*, or, for stages before this has appeared, over the region where it will appear, consequently in the region where we should expect the nervous system to arise, and the region where, according to Oka and Van Beneden et Julin, in *Botryllus* and *Clavelina* it does arise. It is also the region in which more than elsewhere the character of the cells is such that they might most easily be supposed capable of giving origin to new cells. As already shown, the cells are here cuboid in form and have round nuclei and a considerable quantity of cell protoplasm. In all other regions, on the contrary, the cells are flattened, as are their nuclei also, and their protoplasm is relatively less in quantity. The first part of the objection is more weighty than the second, but even this must, I believe, yield to the facts. We shall allow that it

is not sufficient to prove that the ectoderm is *not giving off cells* into the body space *at the time when the Anlage is being formed*. It must also be shown that the process does not take place at *any earlier time*. But when it is considered that the *Anlage* is formed at a very early stage in the life of the bud, it will be seen not to be a matter of great difficulty to examine a complete series of stages from the very inception of the bud up to the time when the *Anlage* is fully formed. This I have done repeatedly, that is, on numerous series of sections, and the description of the ectoderm already given applies as well to one stage as to another. To emphasize this fact I would again call attention to Figs. 68 and 74, *ec.*, the first from a bud in which the *Anlage* has not yet appeared, the second from one in which it is forming. I thus hope to have successfully assumed the risk of *positively denying that the nerve ganglion comes from the ectoderm in the blastozoids of Perophora*, even though I cannot be so positive as to what its source really is. Concerning the difficulties in the way of deciding whether the inner vesicle or the "mesenchyme" cells are the real source of the *Anlage*, I need only refer the reader to what has already been said about the difficulties involving the study of the origin of the pericardium. The well-nigh universal distinctness of the line of separation between the *Anlage* and the wall, the lack of compactness of the *Anlage* in its early stages, and the close resemblance of its cells to many of the surrounding blood cells, here, in the same degree as there, suggest the latter as being the source of the *Anlage*. Fig. 72, Pl. XVII, affords a typical illustration of this. It is drawn from the section in which, if in any one of the series, the separating line is interrupted by cells passing from the wall to the *Anlage*. At the middle point of the separating line there seems to be such an interruption.

By far the most convincing evidence that I have of the origin of the *Anlage* from the vesicle consists of the occurrence in a single specimen of what is almost certainly *an imperfect evagination by which it is formed*. Fig. 74, Pl. XVII, represents the section in which this is most clearly seen. There is no doubt that the separating line is interrupted here at the point

gl.ev., which I believe to be the evagination; and there is also no doubt that the cells of the *Anlage* are continuous with those of the vesicle wall. While it is true that, as shown by the figure, the evagination is not clearly defined either as to its cellular wall or as to its cavity, I am unable to see any valid objection against regarding it as such; the chief difficulty, so far as I see, lies in the fact that the instance is an isolated one. It is impossible that such a condition can be general, since, as already said, I have seen it in a single bud only among the large number examined of practically the same developmental stage. It may be asked if we have not here a rather late stage of development in which the mouth of the hypophysis is already formed; and if the opening here figured does not represent it. But when the entire series of sections is examined and compared with those from younger and older buds, there is really no ground for question on this point. The hypophysis mouth is not formed till a considerably later time, and it is always a well-defined opening, but smaller than this. It is my strong belief that originally the *ganglio-hypophyseal Anlage* arose from the inner vesicle by an evagination in *Perophora*, just as it does in *Goodsiria*, *Botryllus*, *Glossophorum*, and *Distaplia*. For some unaccountable reason the process has undergone secondary modification, till in most cases, in *P. annectens* at least, the evagination has been wholly replaced by a cell proliferation. Occasionally, however, the earlier evaginate process is reverted to; and such a case is presented in the individual shown in Fig. 74. The individual variation here seems to be somewhat similar to that which occurs in the gastrulation of *Aurelia flavidula*, where it has been recently shown, Miss Hyde ('94), that in some instances the endoderm is formed by a regular invagination, while in others it is formed, in part at least, by delamination and by inwandering of the blastosphere cells.

The completion of the hypophyseal duct and the differentiation of the ganglion from its dorsal wall takes place relatively later here than in *Goodsiria*. It will be remembered that in the latter species the duct is well formed, and the ganglion entirely separated from it at a stage when the peribranchial sacs are but just begun and the endostyle is still scarcely

indicated. In *Perophora*, on the other hand, the ganglion is not fully separated from the duct till the peribranchial sacs are well developed, and the stigmata have begun to form. Likewise the endostyle is well advanced to its final form.

The first differentiation that occurs in the solid, irregular cell mass, that at first constitutes the *Anlage*, consists in the modeling of this into a quite regular cylinder, in which there soon appears a lumen. The wall of the tube thus formed is several cells thick, and is of about equal thickness on all sides throughout its length. Before the lumen is wholly completed a fusion between the walls of the anterior end of the tube and of the branchial sac takes place, the fused area becomes perforated, and thus the hypophysis mouth is produced. The formation of the ganglion begins by a rapid proliferation of cells in the dorsal wall of the duct. Nearly the entire length of the duct participates in the process, although in the nearly adult condition the ganglion does not extend entirely to either end of the duct. Fig. 76 represents a transverse section of a duct at an early stage in the development of the ganglion, and Fig. 75 a longitudinal section of a much later stage — a stage, in fact, when the ganglion is almost completely separated from the duct.

The intervening stages I have not thought it necessary to figure. They occur in many of my sections, however, and the whole process is very clear, and easily observed. The separation takes place considerably earlier in some buds than in others, and in a majority of cases it is completed before a stage so late as that shown by Fig. 75.

I may, perhaps, here refer again to the fact that the ganglion in this species develops on the dorsal side of the duct, while it develops on its ventral side in *Goodsiria*.

It is unnecessary to follow the development further. In a short time the ganglion reaches a diameter considerably exceeding that of the duct, and it acquires the characteristic mantle of ganglion cells.

A single developmental point has been omitted that ought, perhaps, to be mentioned. In a few individuals I have noticed a distinct thickening in the *ventral wall* of the tube. Where

this has occurred most conspicuously it has been before the appearance of the ganglionic thickening in the dorsal wall. It is possible that this may be an embryonic trace of the glandular portion of the hypophysis that is so well developed in some Ascidians. There is no particular evidence for this, but the fact that this structure is known to undergo degeneration in some Ascidians gives the suggestion some probability.

In my description of *Perophora annectens* ('94) I have called attention to the fact that the posterior ends of the ganglion and duct are not situated in the median plane of the body, but are considerably to one side. This condition is assumed early in the development of the organs, and is almost always quite pronounced, but I do not see that it has any particular significance.

C. GENERAL CONCLUSIONS AND REFLECTIONS.

I. APPLICATION OF GENERAL DEVELOPMENTAL PRINCIPLES TO AN INTERPRETATION OF THE FACTS.

It being now, as I believe, fully established that the nervous system of the Ascidian bud has a different origin from that of the embryo, we must seek for an explanation for so anomalous a fact, — for the *processes* of evolution are of more importance from a scientific point of view than are their particular *products*.

The explanation, which I think to be the true one, has already been pointed out, first by Seeliger ('85), and more recently by Hjort ('95). The latter, in particular, has dwelt quite fully upon the point. It is, however, a matter of such importance that I believe it deserves to be emphasized still further. This is an instance where nature herself has performed an experiment in modifying the ordinary course of development. The revolutionary and comparatively crude, but still, in considerable degree, successful efforts of experimental embryologists to artificially divert cells that would normally become ectoderm into entoderm, or structures ordinarily of endodermic origin, have been deservedly held to be of the highest moment for solving the fundamental problems of animal development. Cer-

tainly, then, if cases can be found where nature, with her conservative and infinitely delicate methods, has entered upon the same general line of experimentation, and has carried her efforts through to complete success, such cases cannot be too carefully studied.

Let us observe with attention the state of things in the Tunicate bud. The ectoderm is part and parcel of the ectoderm of the parent (this is strictly true in forms like *Goodsiria* and *Botryllus*, where no stolon is present ; and is also essentially true when the budding is stolonial, since here the ectoderm of the stolon is only a prolongation of that of the parent). This is equivalent to saying that the ectoderm of the bud is not an *embryonic structure* at all. It is, on the contrary, a *differentiated organ*. Its function in the parent is to secrete the cellulose test, and in the bud from the very earliest stage it has the same function. The specialization of this secretory function must be deep-seated, for, as Hjort has pointed out, the cellulose character of the test necessitates this. Furthermore, not only is the specialization deep-seated, but also there must be a great and constant activity of the cells ; for not only is the test considerable in quantity, but it must be perpetually produced through the whole life of the zooids to replace the continual waste that is taking place from the external surface. One not infrequently finds great quantities of diatoms embedded in the surface layers of test, and it is well known that many species of animals, particularly small Crustacea, work their way into the test of Ascidians and there lead a semi-parasitic life. Even where no foreign organisms were present, I have often observed the surface test in various species to be eroded and ragged.

The ectoderm, then, has a well-established physiological function to perform in the bud from its very earliest stage of development. How is it with the endoderm ? It is scarcely possible to see how a structure could be more favorably situated for retaining, so far as its functional relation to the organism as a whole is concerned, an undifferentiated character than is the "endoderm" in the early bud. Not only is it wholly protected from contact with the external world, it being enclosed in the ectodermic vesicle, but it has nothing to do in the preparation

of its own food, for it is entirely bathed in the maternal blood. It is relieved from all offices except to assimilate already digested food, and to grow.

Why should not the production of structures which in the embryonic development belongs to the ectoderm be here transferred to the endoderm? And so it is. This conclusion is the more justified when it is considered how different are the conditions under which the two layers develop in the embryo. Here the neural canal is formed while yet the ectoderm is strictly an embryonic structure, and before the endoderm (particularly in the compound Ascidians, *e.g.* *Amaroecium*, Maurice et Schulgin, '84) is differentiated from the richly laden yolk cells which ultimately give it origin.

We have here an instance where physiological requirements have run counter to the course in which, through heredity alone, development would proceed; and the former have proved more powerful. To my mind the chief difficulty in such a case is not that developmental conditions can so profoundly modify the usual course of things, but that by such different courses precisely the same result should be reached. In *Perophora*, for example, no one has detected any difference between the adult embryozoid and the adult blastozoid. The same is true of *Clavelina*, and this case is more important, perhaps, because the embryology of this genus has been very thoroughly studied up to the practically adult state.

It appears certain that heredity has here an *ultimate aim*, as we may call it, and that it is able to reach this even though it be thrown considerably off its regular course by special functional requirements; in other words, that heredity is prospective.

In this connection one may refer to the fact that in some compound Ascidians, *e.g.* *Botryllus*, there is no such thing as an adult embryozoid, and the suggestion may be made that the much abbreviated life of the embryozoid in such instances is in some way correlated with the profound modification undergone in the development of the blastozoid as compared with the embryozoid.

I have already pointed out on another occasion ('95a) that at least one other instance of anomalous bud origin may receive

a physiological explanation somewhat similar to that above supported as the cause of the course of things in the Ascidian bud. I refer to the ectodermal origin of the bud in *Rathkea octopunctata*, as recently described by Chun ('95). The buds of this medusa develop on the manubrium, and on that portion of it in which the endoderm cells, as is clearly shown by the author's description and figures, are highly modified for the digestive function. On the other hand, the ectoderm cells are as clearly much less highly modified. When we look for a reason why the ectoderm retains to so considerable a degree its undifferentiated character, we find a sufficient one in the fact that it is largely relieved of the protective function that usually belongs to this layer in adult animals by its being itself well protected by the deep, close sub-umbrella of this medusa. And in the two facts that the buds arise in a region where the endoderm is highly modified for the digestive function, and that the ectoderm remains comparatively unmodified, appear, as I believe, an adequate reason why the ectoderm alone contributes to the formation of the buds.

Braem ('95), in puzzling over this case, suggests that the most probable explanation of the anomalous condition is to be found in the fact that the buds arise from the same layer, and not remote from the position where the sexual cells are produced. But Chun states, page 32, that "Mann trifft keine Sporen oder parthenogenetischen Eizellen an, welche durch Grösse und abweichendes Verhalten des Inhaltes sich von den übrigen Ektodermzellen abheben."

Of course, if my explanation of this case is correct, we may expect to find other instances where similar conditions and causes will have produced like results ; as, for example, in the budding medusa of *Bougainvillia Niobe* recently described by Mayer ('94), which is likewise a deep-belled form, and in which the buds "spring from the gastric region manubrium." But I do not think that if in a particular instance, where the conditions are right to produce the results, they still do not appear, we should on that account be justified in concluding their inefficiency to produce them. It is highly probable that, in groups of animals which reproduce by budding, the faculty is

acquired independently by different species and at different times.

Now it is certain, both on theoretical and on observational grounds, that there would always be a tendency, and a strong one, for all the germ layers to participate in the production of the bud ; and should they be found to do so in particular cases where the conditions are such as to cause, according to my idea, one or the other of the layers to be excluded from the process, this might be due to the fact that such exclusion had not yet been fully effected because of the comparatively recent acquisition of the property of budding.

2. ON THE QUESTION OF DIFFERENT TYPES OF BUDDING AMONG TUNICATES.

Having now before us the facts concerning the budding in these two genera, we must compare them in order to see whether the differences must be regarded as fundamental, or whether the mode of development in the two cases is more probably a modification of a common type.

The first point to be considered is the relation of the bud to its parent. Various authors have expressed more or less positively the view that the two modes of bud origin represented by *Perophora* and *Goodsiria*, *i.e.* the stolonical and the pallial, have been derived from a common primitive type. Garstang ('95) is the most recent of these, and he has adopted this interpretation apparently with considerable confidence.

It is interesting to notice that the relation between the blastozooids of a colony in these two genera may be viewed in such a way as to give the appearance of a rather close resemblance.

If we disregard the embryozooid from which the colony has sprung in each case, and fix our attention upon the adult blastozooids only, we may reduce the entire colony to a series of individuals connected with one another by their peribranchial sacs through the stolonical septum, or cloison.

This is evident enough in *Perophora*, where it is essentially the actual state of things ; the only modification of it being

that in some instances at least, as already shown, the connection between the zooid and the stolonial septum becomes severed.

To see how the same relations would be produced in *Goodsiria*, it is only necessary to imagine the bud to retain the connection with its parent, and for the connecting neck, shown almost severed in Fig. 15, Pl. XII, to become more elongated; in other words, to form a stolon.

Text figures 1 and 2 illustrate the scheme that is here described, 1 representing *Goodsiria* and 2 *Perophora*; *d* indi-

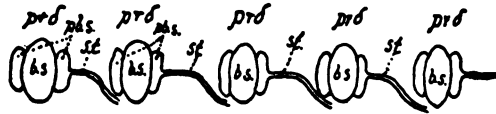


FIG. 1.

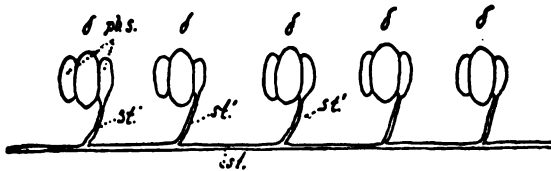


FIG. 2.

cates daughter and *p* parent; *b.s.* branchial sac, *pb.s.* peribranchial sac, and *st.* stolon. The stolons in *Goodsiria* are not connected to the daughter zooids, since we do not know whether the connection would be to the branchial or peribranchial sacs.

But when we come to examine the subject more closely we find that the likeness of the two conditions is much less strong than it at first appears. In the first place we see that the blastozooids of the *Goodsiria* colony would stand in the relation to one another of mother, daughter, granddaughter, etc., while in *Perophora* this is not so. Here the blastozooids are rather all to be looked upon as sisters; that is, they are all produced by the stolon, and we have no facts to indicate that the stolon ever arises from any zooid excepting the original embryozooid of the colony. Another distinction that results directly from

the one just pointed out is this : in *Goodsiria* it would always be the mother ascidiozoid that would be attached to the stolonial septum by its peribranchial sac, while in *Perophora* it is the daughter zoid that is so attached. Or, to express the same thing in another way, we do not know how any given blastozoid of *Goodsiria* would be attached to the supposed stolon from which it would arise. We only know how it would be attached to the stolon that would arise from *it*.

In reality, then, there is a rather deep-seated difference between the processes in the two genera. I am, however, far from affirming that the difference is fundamental. It is not at all impossible that they may be modifications, though rather profound ones, of a common original process. How is the question to be decided? The answer I wish to give with emphasis. If it is ever to be correctly answered, it must be by considering the evidence afforded by the *blastogenesis in connection with the evidence to be derived from embryogenesis and from the comparative anatomy of the adults*.

It was my original plan to make the discussion of the question as full as the data at hand would permit ; and with this in view I have already prepared the manuscript for the anatomical comparison, and in fact have presented it in brief on another occasion. Further reflection has, however, convinced me that it will be better not to attempt a full description until the embryological data for both genera, now almost wholly wanting, have been furnished. We must know how the peribranchial sacs arise in the embryo in each case, and also how the first bud arises in each case, before we have a basis on which to profitably speculate.

It will be worth while to point out briefly how unsatisfactory such speculation would of necessity be in view of our imperfect knowledge of the ontogeny in these genera.

We have no observations on the origin of the peribranchial sacs in the embryo of either *Perophora* or *Goodsiria*. Neither do we know how the first bud arises in either case. We might infer that in these particulars *Perophora* is like *Clavelina* ; in fact, such an assumption is generally made. But as I have shown the relation of the blastozoids to the stolon to be very

different in the two cases, it seems rather probable, at least not at all improbable, that there may be important differences between them in the two points mentioned, *viz.*, in the origin of the peribranchial sacs and the first buds.

As regards *Goodsiria* we may assume that the embryonic development is essentially like that in *Botryllus*. For my own part I fully expect that this will prove to be the case. However, we are by no means certain of it, and even if we were we should be far from clear sailing as regards the origin of the peribranchial sacs of the embryo, since Pizon insists that they arise from the endoderm of *Botryllus*, and Garstang is inclined to accept his conclusion. If the peribranchial sacs of *Perophora* arise from the ectoderm as they do in *Clavelina*, and *if* the stolonial septum of the first bud of *Perophora* arises from the pharynx as it does in *Clavelina*, and *if* the first bud of *Goodsiria* arises like the first bud of *Botryllus*, and *if* the peribranchial sacs of *Botryllus* arise from the ectoderm, then weighing all the evidence together, anatomical as well as developmental, it seems to me that the budding in the one genus is genetically independent of the budding in the other.

This view I have practically expressed before ('95a), in contending that the Compound Ascidians have had a double origin from the Simple ones.

But, as already said, I regard it as necessary that the above formidable array of ifs should be gotten out of the way before the discussion can profitably be carried farther.

And now concerning the "epicardiac tubes" that are of so much importance in connection with Tunicate budding. I have elsewhere ('95) said, regarding the origin of the pericardial vesicle in *Goodsiria*, that an epicardium is here present "as in *Botryllus*." As will be readily seen by comparing Figs. 18 and 19, Pl. XIII, with, for example, the woodcut given by Pizon ('93), page 30, of *Botryllus*, there can be no doubt that the posterior extremities of the peribranchial sacs in my figures of *Goodsiria* are the same as those marked *p.v.* by Pizon. The only difference in the two cases is in the size. The two pouches are much longer in *Botryllus* than in *Goodsiria*. Both Pizon ('93) and Garstang ('94) regard the structures in *Botryllus*

as homologous with the epicardium of *Clavelina*, *Distaplia*, *Fragroides*, and other species.

Thus the last-named author says: "In spite, therefore, of the final difference of position between the epicardial (or perivisceral) sacs of the Botryllidae and the epicardial tubes of *Distaplia* or *Clavelina*, there can be no doubt, as Pizon has maintained, that there is an exact homology between the two structures." Nevertheless, there is, in my mind, a very grave doubt that the structures in *Goodsiria*, which are certainly the same as those called epicardial sacs in *Botryllus*, have anything whatever, either morphologically or physiologically, to do with the epicardial tubes of *Clavelina*.

It is well known from the investigations of Seeliger and Van Beneden et Julin that the epicardial tubes of *Clavelina* arise from the branchial sac, and they arise in the same way in *Glossophorum* (Hjort, '95) and *Fragroides* (Maurice, '88). As Pizon and Garstang contend, the mere fact that the structures arise from the branchial sac of some species, and from the peribranchial sacs in others, would not in itself present any difficulty against regarding them as *genetically homologous* (and I take this to be the kind of homology that these authors mean, for I do not suppose they recognize any other kind). But this view would have to regard it as proven that the branchial and peribranchial sacs both arise from the endoderm in the embryozoid; and this can certainly not be granted as our knowledge now stands.

As previously said, Pizon claims that such is their origin in the *Botryllus* embryo; and Garstang is inclined to support the same view. Should it finally be established that this is in reality the case, then, so far as this much of the evidence is concerned, the structures may be homologous in *Botryllus* and *Clavelina*, and of course in *Goodsiria*, also, if its embryonic development here is similar to what it is held by Pizon to be for *Botryllus*; but this is still to be shown.

But now it being conceded that, so far as concerns the evidence yet in hand relating to the origin of these structures, they may *possibly* be homologous, we must still consider what the evidence of their destiny is.

In *Clavelina*, *Distaplia*, *Glossophorum*, and *Fragroides* the epicardium gives origin either directly or through the stolonial septum to the inner vesicle, or endoderm, of the bud. In *Botryllus* and *Goodsiria* the so-called epicardial sacs have nothing whatever to do with the budding, and there is not the slightest evidence that they ever had anything to do with the process. The last part of this statement is at least true for *Goodsiria*. Here the buds arise, as reference to Figs. 9 and 10, Pl. XII, will show, far forward on the parent zooids, while the epicardial pouches are at the extreme posterior end of the animal. All they are is this: When the intestine begins to form at the postero-ventral side of the primitive inner vesicle it produces, as one might say, an obstacle in the way of the further expansion posteriorward of the inner vesicle, which, of course, is constantly growing. The notch thus produced has been already described, and is well seen in Fig. 16, *d*., Pl. XIII.

The extensions of the vesicle on each side of this notch are the epicardial sacs of the adult animal. When the peribranchial sacs are completely formed the epicardial sacs are merely their posterior extremities.

And now a word concerning the relation of the epicardium to the heart. In attempting to show that the epicardium is the same structure in all Tunicates, and to make use of it as a basis for classifying the group, Garstang has tried to escape the difficulty of its being in some cases connected with the heart, while in other cases it is not, by supposing that such connection is secondary.

In my account of the origin of the heart in *Goodsiria* I have shown that it arises from the right epicardial pouch, and I can certainly see no reason for regarding this method of origin as secondary. In fact, it was partly this consideration that made me speak of the pouch as an epicardium in my preliminary communication.

Concerning the epicardium in *Perophora*, after describing the relation of the bud to the stolon, I have said in my preliminary paper: "It is obvious that there cannot be in *Perophora* an epicardium corresponding to the structure called by that

name in *Clavelina*, since in this species the epicardium is connected with the *branchial* sac."

Lefevre ('95) says: "No epicardium is present; in this respect *Perophora* differs strikingly from *Clavelina* and some other Ascidians." So far as the *blastozooids* are concerned these unqualified statements are, I believe, fully justified. It must, however, be remembered that we do not know how the stolon originates from the embryozoid, and until we are informed on this point I must place a certain reservation on my assertion of the entire absence of the epicardium in *Perophora*.

If we accept Garstang's view that the relation of the heart to the epicardium is secondary, then the fact that the heart arises on one side of the body, while the stolon septum is attached to the peribranchial sac of the other side in *Perophora*, would be of little weight against supposing an epicardium to be present in this species. But I have already shown that this author's conjecture is contradicted by the evidence of *Goodsiria*, if he would still maintain that the pouches described in this species are homologous with the epicardium of *Clavelina*.

In the present state of our knowledge on this point, then, I am a long way from conceding, as Garstang thinks we must, "that these modifications of the epicardial tubes provide a sound basis for a true and genetic classification of Tunicate budding." That, *when considered in connection with various other developmental and anatomical facts*, it is of prime importance in interpreting the budding, cannot be questioned. But the attempt to make it, in itself, a basis for classifying the Compound Ascidians can hardly be more satisfactory than one-legged classifications ever are in zoölogy.

D. SUMMARY OF RESULTS.

GOODSIRIA DURA.

1. The budding is pallial, and the buds arise far forward on the parent zooid. In no case has more than one bud been seen on the same parent.

2. No "budding zone" is recognizable in zooids on which buds are not developing.

3. The buds become wholly separated from the parent zooids at a very early stage, *i.e.* before any differentiation of organs begins; and at a considerably later time they become secondarily connected with the vessels of the test.

4. The ampullae of the testicular vessels do not produce buds.

5. The formation of the branchial and peribranchial sacs, and of the digestive tract from the primitive inner, or endodermic, vesicle of the bud does not differ in any essential particular from that of all other Compound Ascidians.

6. The common *Anlage* of the pericardium and heart is derived from the wall of the endodermic vesicle by an imperfect evagination that does not become fully separated until the ventral partitioning folds which separate the peribranchial sacs from the branchial sac have reached back to the region where the heart is forming.

7. The ganglio-hypophyseal *Anlage* arises as a gutter-shaped evagination from the dorsal side of the endodermic vesicle. As this closes off to become the hypophyseal duct, the ganglion is produced from the mass of cells that forms the last of the connection of the duct to the endodermic vesicle. The ganglion, therefore, in this species as in *Botryllus*, lies *ventral to the hypophyseal duct*.

8. The youngest sexual cells observed were found free in the body space of the buds, so that in all probability they pass from parent to bud as is the case in *Botryllus*. But in none of the material available for study were the elements present in sufficient quantity and development to make it possible to give a complete history of them. The "polycarps" appear to

be confined to a single row on each side of the endostyle, and not far from it.

PEROPHORA ANNECTENS.

1. The inner or endodermic vesicle of the bud is derived from the stolonial septum; and from this are derived the branchial and peribranchial sacs, and the digestive tract in a manner in all essentials similar to that of all other Ascidian buds.

2. The peribranchial sacs of the developing blastozoid are formed in such a manner that the stolonial septum is connected to the left one of these, and not to the branchial sac, as has been hitherto supposed.

3. The pericardial *Anlage* arises almost certainly from the wall of the inner vesicle, though there remains some doubt whether or not it may be produced by an aggregation of mesenchyme cells. But if this is so it is still probable that its ultimate source is the endoderm, since the mesenchyme cells that seem to enter into it are themselves probably produced by the endoderm.

4. The ganglio-hypophyseal *Anlage* was originally produced from the endodermic vesicle as an evagination, as it is in the buds of numerous other Ascidians. This primitive method of origin is, however, found in an occasional individual only at the present time, the evagination having, in most cases, been replaced by a migration of cells.

For the most part this migration takes place at the point at which the ganglion will be ultimately situated; but it may occur at other points more or less remote from this, and the coming together of these migrating cells makes it appear as though the *Anlage* is produced from mesenchyme cells. Or, as in the case of the pericardial *Anlage*, we may consider that mesenchyme cells do participate in the formation of the *Anlage*, but that these cells are derived from the endodermic vesicle.

IN GENERAL.

1. It is now established beyond question that in some, at least (and *Goodsiria* may be taken as one of the best instances of this), of the Compound Ascidians the outer layer of the bud contributes much less to the structure of the adult blastozooids than it does to the adult embryozooids. This is most conspicuously seen in the case of the nervous system, for this is certainly produced from the outer, or ectodermic layer, of the embryo, while it is as certainly produced from the inner layer of the bud. Whether we call this inner layer endoderm or not, the fact of chief importance remains that the same layer produces most of the organs of the zooid, among which are included the digestive tract and the nerve ganglion.

2. The anomalous course of development of the bud is due to the fact that the ectoderm is at no time in the life of the bud an undifferentiated, or embryonic layer. It is from the very outset and always a fully formed organ, its function being to secrete the cellulose matrix of the test. The inner, or endodermic, vesicle of the bud is, on the other hand, in the completest sense, an undifferentiated, or embryonic layer. By this purely physiological cause the inner layer has been made to produce structures, most important of all the nervous system, which in the embryo are produced by the ectoderm.

3. Illustrations of the potency of physiological influences to profoundly change the usual course of development are found in the budding of other animals, one of the most instructive of which is the medusa *Rathkea octopunctata*, where the change has been in the opposite direction from that in the Ascidians; for here the endoderm takes no part in the formation of the bud, the whole blasto-medusa being produced from the maternal ectoderm.

4. The evidence now in hand, drawn from adult structure and from the blastogenesis strongly tends to the conclusion that the budding of *Goodsiria* and *Botryllus*, represents a type that is genetically independent of the type represented by *Perophora*. In other words, that the type of budding represented by *Goodsiria*, has originated independently of the

type represented by *Perophora*. But more knowledge of the embryology of each genus is requisite before this or any other conclusion respecting their relationships will be fully warranted.

5. In neither *Goodsiria* nor *Perophora* is there an epicardium comparable with the structure called by that name in *Clavelina* and many other Compound Ascidiæ.

6. The budding of *Goodsiria* greatly strengthens the conclusion justified by adult structure that the Polystyelidae and Botryllidae are very closely related. The two families will probably have to be ultimately united into one, but it is not best to do this until we know the embryology of both groups much more fully than we yet do.

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Nov. 11, 1895.

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DESCRIPTIVE LETTERS.

<i>a.</i>	Anterior.	<i>int.</i>	Intestine.
<i>amp.</i>	Ampullae of testicular blood vessels.	<i>in.ves.</i>	Primitive inner vesicle.
<i>at.sip.</i>	Atrial siphon.	<i>i.p.</i>	Internal papilla.
<i>bd.</i>	Bud.	<i>l.coec.</i>	Lacteal coecum.
<i>bd.a.</i>	Bud <i>Anlage</i> .	<i>l.d.</i>	Lacteal duct.
<i>b.c.</i>	Blood corpuscles.	<i>l.pb.s.</i>	Left peribranchial sac.
<i>br.s.</i>	Branchial sac.	<i>l.s.</i>	Lacteal system.
<i>br.sip.</i>	Branchial siphon.	<i>m.en'c.</i>	Male polycarp.
<i>br.sta.</i>	Branchial stigmata <i>Anlage</i> .	<i>mes.gas.</i>	Gastric mesentery.
<i>br.s.ep.</i>	Branchial epithelium.	<i>mes.rec.</i>	Rectal mesentery.
<i>br.st'g.</i>	Branchial stigmata.	<i>oe.</i>	Oesophagus.
<i>b.s.</i>	Body space.	<i>ov.</i>	Ova.
<i>b.v.</i>	Blood vessel.	<i>p.</i>	Posterior.
<i>cl.</i>	Cloaca.	<i>pb.s.a.</i>	<i>Anlage</i> of peribranchial sac.
<i>cl'n.</i>	Cloison, or stolonian septum.	<i>pc.</i>	Pericardium.
<i>cl.ep.</i>	Cloacal epithelium.	<i>pc.a.</i>	Pericardial <i>Anlage</i> .
<i>d.</i>	Dorsal.	<i>p.f.</i>	Partitioning fold.
<i>d.f.</i>	Dorsal partitioning fold.	<i>p'l'c.</i>	Polycarp.
<i>d.l.</i>	Dorsal lamina.	<i>r.c.</i>	Remnant of connection between bud and parent zooid.
<i>ec.</i>	Ectoderm.	<i>rec.</i>	Rectum.
<i>ec.ves.'</i>	Ectodermic, or testicular blood vessels.	<i>r.e.o.</i>	Remnant of evagination.
<i>ec.ves.'</i>	Ectodermic vessels projecting into body space.	<i>r.pb.s.</i>	Right peribranchial sac.
<i>end.</i>	Endostyle.	<i>r.v.f.</i>	Right ventral partitioning fold.
<i>en'c.</i>	Endocarp.	<i>s.ec.</i>	Siphonal ectoderm.
<i>ep.pb.s.</i>	Epithelium of peribranchial sac.	<i>s.f.</i>	Siphonal folds.
<i>f.en'c.</i>	Female polycarps.	<i>st.</i>	Stomach.
<i>gl.</i>	Ganglion.	<i>sto.</i>	Stolon.
<i>gl.ev.</i>	Ganglionic evagination.	<i>t.</i>	Testa.
<i>ht.</i>	Heart.	<i>t.c.</i>	Testa cells.
<i>hy.a.</i>	Hypophysis <i>Anlage</i> .	<i>tr.ves.</i>	Transverse vessel.
<i>hy.d.</i>	Hypophysis duct.	<i>ts.</i>	Testis.
<i>i.l.b. 1,</i>	Internal longitudinal bars.	<i>v.</i>	Ventral.
<i>i.l.b. 2,</i>		<i>v.f.</i>	Ventral partitioning fold.
<i>i.l.b. 3,</i>			
<i>i.l.b. 4,</i>			
<i>i.l.b. 5,</i>			

EXPLANATION OF PLATE XII.

GOODSIRIA DURA.

The figures were drawn with the aid of a camera lucida and a Leitz microscope, excepting when otherwise specified.

FIG. 1. A colony, natural size, completely covering the surface of a piece of seaweed. No buds are present in this colony. Not camera, but as faithful a reproduction as possible.

FIG. 2. A colony, also on a piece of seaweed. The Ascidiozooids much less closely crowded than in the preceding. Buds in various stages of development are seen, and some of the very young ones are not as near the margin of the colony as are some of the older ones. The ampullae of the testicular vessels are seen, particularly near the margin of the colony. Not camera, but as exact as possible. $\times 4$.

FIG. 3. Small fragment of a thin colony removed from its substratum and examined as a transparent object in clove oil. $\times 87$.

FIG. 4. Digestive tract with a portion of the branchial sac attached. The longitudinal folds of the stomach and the single spiral groove of the intestine are seen. $\times 87$.

FIG. 5. Camera sketch of a section of a colony showing sections of three blastozooids in various stages of development. The top of the figure corresponds to the top or dorsal surface of the colony; it is consequently seen that the blood vessels of the test, *ec.ves.*, are at a deeper level than are the Ascidiozooids. $\times 87$.

FIG. 6. Transverse section of an almost fully formed blastozooid, the section being well toward the posterior end of the body. $\times 120$.

FIG. 7. An optical section of two ampullae of the testicular vessels which contain a closely packed mass, *b.c.*, of blood corpuscles. This, together with the thick ectodermal wall of these vesicles, give them somewhat the appearance of young buds. Drawn from a specimen cleared in clove oil. $\times 87$.

FIG. 8. A single male "polycarp" attached to the mantle. The short vas deferens is here seen. $\times 120$.

FIG. 9. Optical section of an ascidiozooid with a bud, *bd.*, still attached to it. This is the zooid from which the section shown in Figs. 12 and 13 were cut. The direction of the section is indicated by the line *A.A.*' The specimen was cleared in clove oil. Not camera, but as faithful a representation of the object as possible.

FIG. 10. Optical section of a zooid with a young bud, *bd.* Cleared in clove oil. $\times 87$.

FIG. 11. Section of the earliest stage seen in the formation of a bud. As seen, the ectoderm is not yet modified at the point where the bud is forming, the bud *Anlage*, *bd.a.*, being confined to the parietal wall of the peribranchial sac; *br.st.a.* are points where stigmata of the present zooid will form. $\times 210$.



EXPLANATION OF PLATE XIII.

FIG. 12. Section of the budding zooid shown in Fig. 9, the section corresponding to the line *A.A.* × 87.

FIG. 13. From the same series as 12, but far enough posteriorward so that the inner layer of the bud is not continuous with the maternal endoderm. At *amp.* are seen two ampullae of the testicular vessels which are in close contact with the young bud, and the vessel, *ec.ves.*, is still more closely pressed by the bud. × 210.

FIG. 14. Transverse section of a bud not yet separated from its parent. × 210.

FIG. 15. Section of a zooid with a bud, *bd.*, almost severed, there being only a remnant, *r.c.*, of the connection between bud and parent. The bud is here entirely undifferentiated as to the organs of the future zooid.

FIG. 16. Optical section of a young bud; the intestine, *int.*, is established, and the folds that will ultimately separate the peribranchial sacs from the branchial sac, are barely indicated at *p.f.* The ganglio-hypophyseal evagination, *hy.cl.*, is represented as projected on the plane of the section. In reality it would not be seen at the focus at which the rest of the bud is shown. The beginnings of the two so-called epicardial pouches will be observed at *X*. Seen from the dorsal side. × 87.

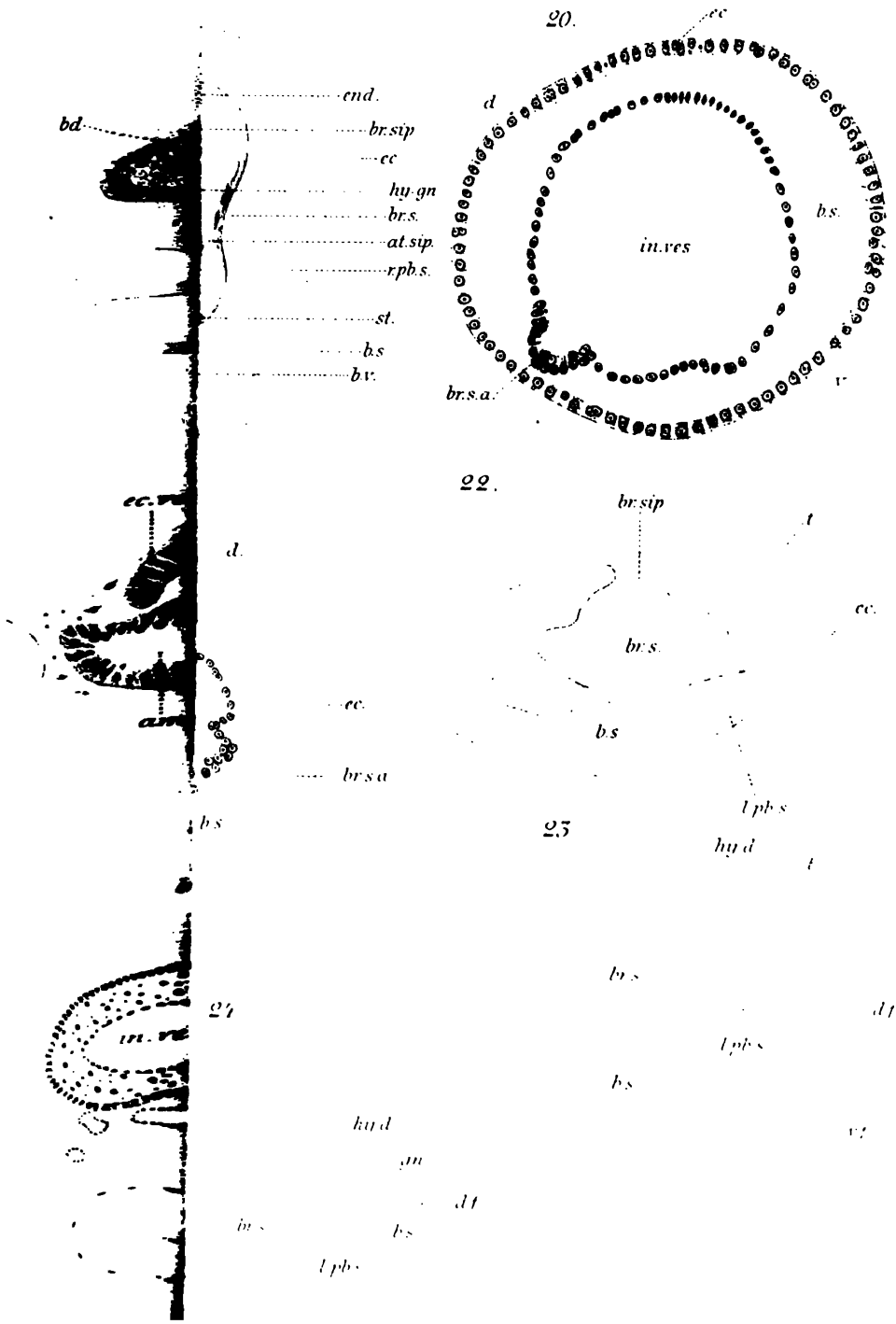
FIG. 17. A bud somewhat more advanced in development than the preceding. The specimen was cleared in clove oil. It is seen from the dorsal side, but is slightly rotated to the right. The right partitioning fold, *p.f.*, is well seen. The distribution of the blood cells in the body space is shown. × 87.

FIG. 18. A bud considerably more advanced than the last, the peribranchial sacs nearly complete. Seen from the dorsal side. Viewed as a transparent object, but not shown in optical section. × 87.

FIG. 19. A still older bud, the differentiation of the organs almost complete. Method of treatment and view similar to the last three. × 87.

FIGS. 20 and 21. Sections of a bud in which the peribranchial sacs, *br.s.a.*, are barely begun. The sections are not quite at right angles to the antero-posterior axis of the bud, and as a consequence the sacs do not both appear on the same section. × 210.

FIGS. 22-24 (and 25, Pl. XIV). Four transverse sections of a bud about corresponding in its stage of development to the bud shown in Fig. 17. Fig. 22, the most anterior of the series, passes through the position at which the branchial siphon, *br.sip.*, is soon to form. By Fig. 23 it is seen that the folds which are to separate the branchial from the peribranchial sacs extend along the ventral as well as along the dorsal side of the primitive inner vesicle, *v.f.* and *d.f.* In the last of the four sections, Fig. 25, no trace of the folds appears. This section passes through the beginning of the atrial siphon, *at.sip.* × 210.



EXPLANATION OF PLATE XIV.

FIGS. 26-29. A series parallel to the last, but from a more advanced bud. The stage of development is about midway between the buds shown in Figs. 17 and 18. $\times 210$.

FIGS. 30 and 31. From the posterior end of a bud nearly as far advanced as the one shown in Fig. 19. The sections show that the branchial sac, *br.s.*, is wholly closed off from the peribranchial sacs, *r.pb.s.* and *l.pb.s.*, and Fig. 31 shows that the peribranchial sacs unite behind the branchial sac to form the wide atrium, *at.* $\times 87$.

FIG. 32. Shows the relation of the digestive tract to the peribranchial sac before it has become pushed into the latter. $\times 120$.

FIG. 33. Shows the digestive tract pushed fully into the peribranchial sac. $\times 210$.

FIG. 34. Section of the digestive tract in the practically adult condition. The lacteal coecum, *l.co.* (this lettering should point to the smaller of the two circular structures; the lithographer has moved the letters), the lacteal system, *l.s.*, and spiral fold of the intestine, pointed to by the index line, *int.*, are seen. $\times 210$.

FIG. 35 (and 36, Pl. XV). Two sections showing stages in the development of a siphon. Fig. 35 shows that the siphon begins by an evagination of the endodermic vesicle. $\times 508$.

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EXPLANATION OF PLATE XV.

FIG. 36. A much more advanced stage; shows the peculiar folding, *s.f.* and *s.f'*, that is produced before the siphon is complete. $\times 210$.

FIG. 37. Shows two of the testicular vessels, *ec.ves.*, projecting into the body space, *b.s.*, of the developing bud. $\times 210$.

FIG. 38. Shows two vessels, *ec.ves.*, opening into the body space. $\times 210$.

FIGS. 39-42. Relate to the origin of the pericardial vesicle. Fig. 39 shows a transverse section passing through the posterior portion of the animal, and at *r.p.b.s.* is seen the pouch of the right peribranchial sac (the epicardial pouch) from which the pericardial vesicle is produced. Fig. 39, $\times 210$; the others, $\times 508$.

FIG. 43. Cross-section of a bud in about the same stage of development as the one shown in Fig. 16. The section of the gutter-like evagination, *hy.d.*, of the ganglio-hypophyseal duct, is here very distinct. $\times 210$.

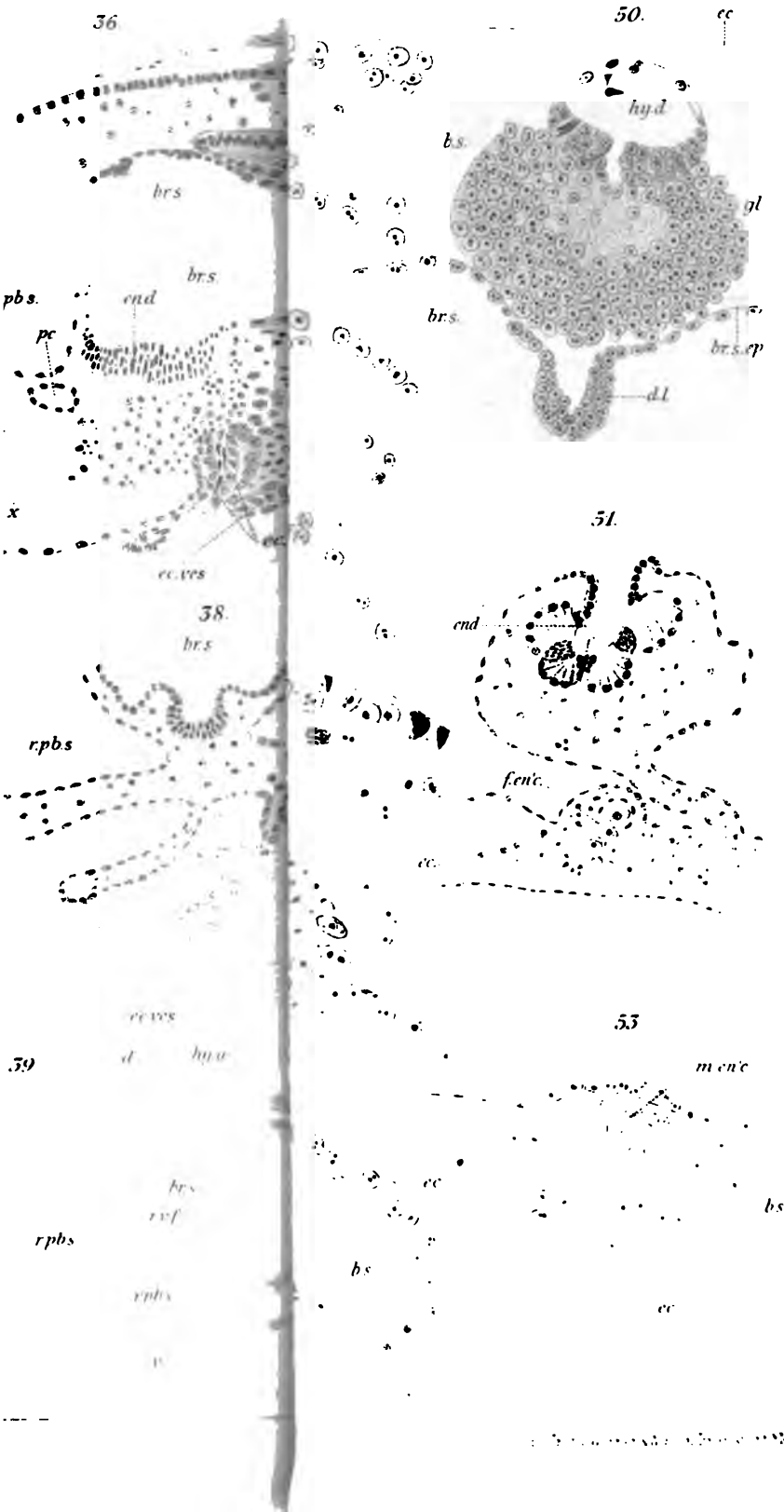
FIGS. 44-47. Four sections of a series passing from before backward of the ganglio-hypophyseal duct. They are almost, but not quite, at right angles to the long axis of the duct. Fig. 44 shows the mouth of the duct, and Fig. 47 shows that at its posterior end, or near the end, the duct still communicates with the branchial sac, *r.c.o.*

Both the ectoderm and the cells in the body space of these sections are reproduced with special care. Figs. 44, 45, and 47, $\times 508$; Fig. 46, $\times 720$.

FIG. 50. Cross-section of the duct and ganglion nearly fully formed. $\times 508$.

FIG. 51. Cross-section of the endostyle showing a young female polycarp. $\times 210$.

FIGS. 53 and 54 (Pl. XVI). Two early stages in the formation of male polycarps. $\times 508$.



EXPLANATION OF PLATE XVI.

FIG. 48. A longitudinal section of the ganglio-hypophyseal duct at a slightly more advanced stage than the one shown in Figs. 44-47. It will be noted that here the communication of the duct with the branchial sac, *r.e.o.*, the remnant of the evagination, is almost closed. The differentiation of the ganglion, *gl.*, from the duct and from the wall of the branchial sac is complete at its anterior end, but not at its posterior end. $\times 720$.

FIG. 49. Cross-section of a duct and ganglion after the ganglion is fully separated, but is still very small. $\times 720$.

FIG. 52. An ovum found alone and free in the body space. $\times 1200$.

FIG. 55. A multinucleated mass floating free in the body space; probably(?) an early stage in the formation of a male polycarp. $\times 508$.

PEROPHORA.

FIG. 56. Small portion of a colony of *P. annectens* almost entirely covering a zooid of *Clavelina*. The specimen is the fully compounded variety, and forms a thin encrusting layer on the surface of the *Clavelina*. The tips of the stolons, *sto.*, and young buds, *bd.*, are shown at the margin of the colony. Not camera. $\times 2$.

FIGS. 57-64. From a series of transverse sections extending from before backward of *P. annectens*, illustrating the method by which the bud is connected to the stolon partition by its left peribranchial sac. The point of connection is seen in Fig. 60. $\times 138$.

FIG. 65. Transverse section of a *P. Listeri* bud, slightly more advanced in development than the preceding; illustrating, likewise, the connection of the left peribranchial sac to the stolon partition. In this section, however, the series passes from behind forward, so that the apparent reversal of right and left does not occur. $\times 138$.

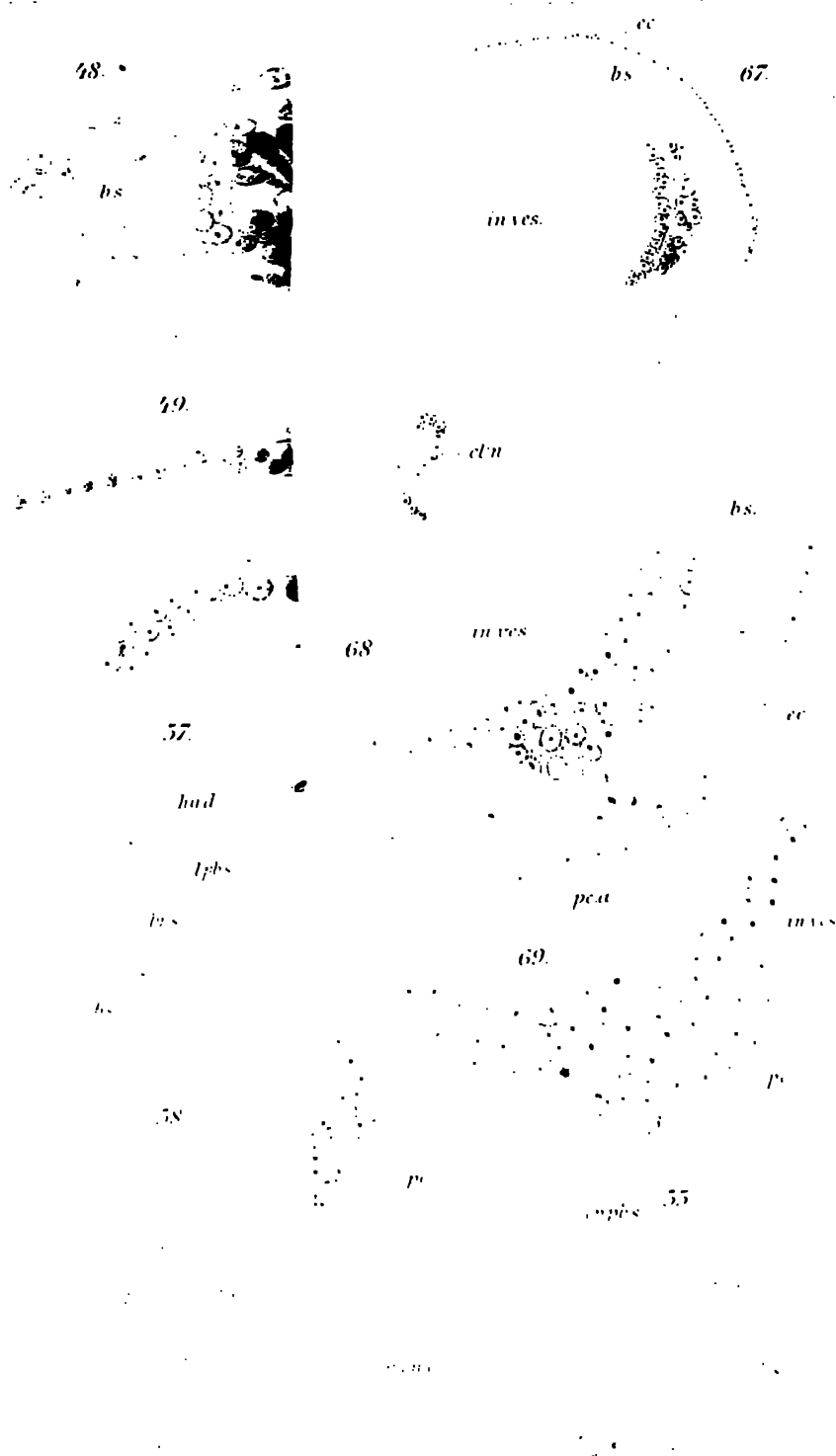
FIG. 66. Shows a case in which the zooid is fully separated from the stolon. This is the section of a complete series in which the stolon approaches most closely to the bud, but here the separation is complete. *P. annectens*. $\times 210$.

FIGS. 67 and 68. Show the development of the pericardial vesicle. Fig. 67 shows its position with reference to the point of attachment of the inner vesicle to the stolon partition. Both are from the same series of sections. *P. annectens*. Fig. 67, $\times 72$; Fig. 68, $\times 330$.

FIG. 69. A later stage in the formation of the pericardium, but still before its separation from the endoderm. $\times 720$.

FIG. 70. From the same series as the last to show the relation of the forming pericardium to the point of attachment of the stolon partition to the primitive inner vesicle. $\times 120$.

FIG. 71. A condition of the pericardium similar to that shown in Fig. 69, but slightly more advanced in development. $\times 500$.



EXPLANATION OF PLATE XVII.

FIG. 72. An early stage in the formation of the ganglio-hypophyseal *Anlage* before its separation from the endoderm. Camera drawing as far as possible with Leitz. oc. 5, obj. 7.

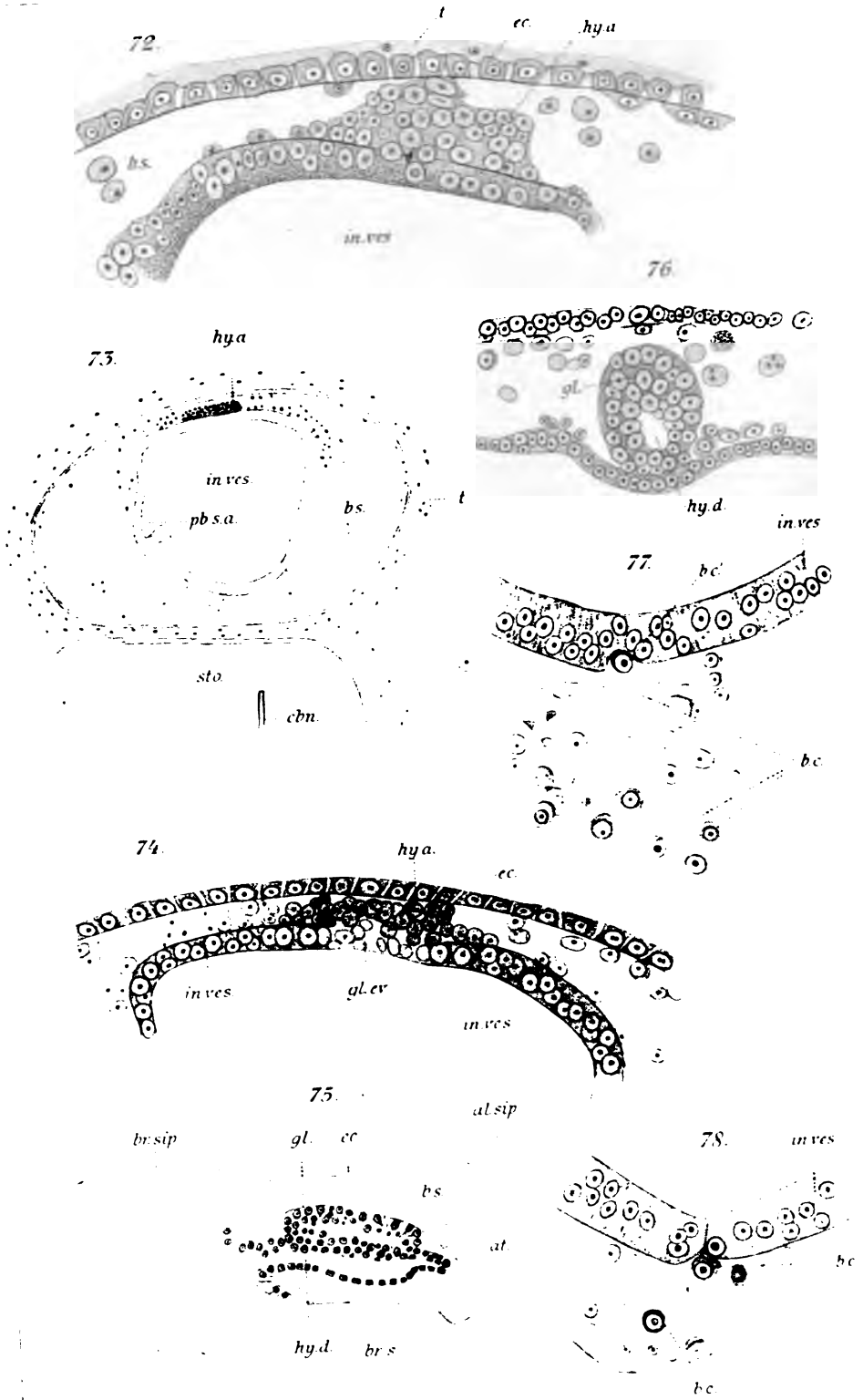
FIG. 73. Shows a stage in the formation of the ganglion somewhat more advanced than the preceding, the whole section drawn in order to show the distribution of the blood or mesenchyme cells in the body space. $\times 210$.

FIG. 74. From the same series as Fig. 73, more highly magnified, showing the supposed evagination to form the ganglio-hypophyseal *Anlage*. $\times 508$.

FIG. 75. Longitudinal section of the ganglion and duct, the former almost separated from the latter. $\times 250$.

FIG. 76. Transverse section of the duct, with its dorsal wall considerably thickened for the formation of the ganglion. $\times 508$.

FIGS. 77 and 78. Two sections in which cells appear to be breaking away from the endoderm, and passing into the body space to become mesenchyme cells. Both of these cases are far from the position where either heart or ganglion will form. $\times 720$.



ON THE SMALLEST PARTS OF STENTOR CAPABLE OF REGENERATION; A CONTRIBUTION ON THE LIMITS OF DIVISIBILITY OF LIVING MATTER.

FRANK R. LILLIE.

IN experiments on the power of multiple development of the ovum the result has been reached, that a portion of less volume than one-fourth that of the normal ovum does not possess the capacity of producing an *embryo* or *larva*, though it may a gastrula (see postscript); while a portion equal to one-fourth or more of the volume of the normal ovum may, under suitable conditions, produce a gastrula and finally a larva of corresponding relative bulk. This result has been attained by Loeb,¹ Wilson,² Driesch,³ Morgan,⁴ and Zoja.⁵ Wilson found only a single larva of *Amphioxus* of one-fourth the normal size and that showed several defects; and Driesch has not, so far as I am aware, mentioned any pluteus of less than one-quarter size. Morgan has recently published the results of his studies on the power of multiple development of the echinoderm ovum. In this he shows that "the volume of the smallest gastrula which can be produced from fragments of the egg falls below $\frac{1}{84}$ the volume of normal gastrulae. The volume of the fragments of the egg which produced such gastrulae, varies between $\frac{1}{40}$ and $\frac{1}{50}$ of the volume of the ovum." (Summary p. 124 *loc. cit.*) But these smallest gastrulae were unable to

¹ Jacques Loeb. "On the Limits of Divisibility of Living Matter." In *Biol. Lectures of Marine Biol. Lab. for 1894*. Boston, Ginn & Co. Also in *Archiv für Ges. Physiologie von Pflüger*. Vol. LIX. 1894.

² E. B. Wilson. "Amphioxus and the Mosaic Theory of Development." *JOURNAL OF MORPHOLOGY*. Vol. VIII, No. 3. 1893.

³ Driesch. "Entwicklungsmechanische Studien." III-VI. *Zeitschr. f. wiss. Zool.* Bd. I.V, p. 9. 1893.

⁴ "Studies of the Partial Larvae of *Sphaerechinus*," by T. H. Morgan, in Roux's *Archiv für Entwicklungsmechanik der Organismen*. Bd. II, H. 1. 1895.

⁵ Sullo sviluppo dei blastomeri isolati dalle uova di alcune meduse (e di altri organismi) per il Dr. Raffaello Zoja; in Roux's *Archiv für Entwicklungsmechanik der Organismen*. Bd. II, H. 1. 1895.

develop further. Morgan himself says (p. 117): "The smallest pluteus which I have found measured 7×8 , and the normal form in the same dish 12×15 . Another larva at the beginning of the pluteus stage measured 6×7 . The larvae have apparently one-eighth the volume of the normal, and correspond in size very nearly to the pluteus figured by Loeb. If, however, we compare these small larvae with the larvae derived from isolated blastomeres, the conclusion is forced upon one that these plutei have in all probability come from fragments of the egg having only about *one-half to one-fourth of the volume of the egg*." Inasmuch as the test proposed for the limits of divisibility rests upon the capacity for complete development to an *embryo*, or *larva* properly so-called, it is only these last figures of Morgan's that demand consideration. It would seem from these that Loeb (*loc. cit.*) has made his figure, *one-eighth*, too low, not having taken in account the fact, emphasized by Morgan, that the growth of the small blastulae, gastrulae and plutei is less rapid than that of the normal.

Zoja's results on the separation of the blastomeres of the ova of certain medusae must also be considered. In the summary, p. 32, we find the following remarks: "Medusae; Die Entwicklung der getrennten Blastomeren ($\frac{1}{2}$ und $\frac{1}{4}$ Ei, von *Liriope mucronata*, *Geryonia proboscidalis*, und *Mitrocoma annae*; $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, Ei von *Clytia flavidula* und *Laodice cruciata*) ist ganz genau in allen ihren Phasen wie diejenige des ganzen Eies." — "Es bildet sich endlich immer eine schwimmende Larva, aus zwei Gewebe bestehend, die von jener, welche aus $\frac{1}{4}$ Ei hervorgeht, nicht unterscheidbar ist, ausser in den Dimensionen."

From this it appears that a $\frac{1}{16}$ blastomere of *Clytia* and *Laodice* is able to develop into a swimming larva. But from the next statement I judge that the development cannot go farther; this is: "Bei *Clytia* zeigten $\frac{1}{2}$ und $\frac{1}{4}$ Ei auch die vollständig entwickelte idroide Form, und bei *Liriope* gab $\frac{1}{2}$ eine kleine runde Medusa, in welcher die vier primären Tentaculi normalerweise im Kreuz angeordnet waren." Thus a fourth blastomere is the smallest portion capable of complete development.

There are three possible explanations of this failure of such small parts to develop:

1. That the whole organization of the species cannot be included in so small a space. Briefly, *deficient organization*.
2. That so small a volume of matter cannot fulfill the mechanical conditions consequent on cell-division, formation of a segmentation cavity, invagination, and so forth, owing perhaps to increased surface tension (Driesch), not to mention other conceivable alterations of the extrinsic factors of development.
3. That such a small part "is not able to set free that amount of energy which would be required for its transformation into a gastrula or a pluteus." (Loeb.)¹

The third explanation seems to me inadequate; because such masses may continue to live for a considerable period of time and display an amount of energy in *atypical* form changes and rapid ciliary movement, which would suffice to produce the phenomena of normal development, did not other factors (included in the first or second of the above alternatives) prevent. Moreover, it is well known that exceedingly minute protoplasmic bodies, very much smaller than one-eighth the echinoderm ovum, may produce a relatively enormous amount of energy: *e.g.* bacteria and spermatozoa. Finally we do not know how much of developmental energy is of intrinsic and how much of extrinsic origin. *We are limited, then, to the first two alternatives.*

Now, in the regeneration of a unicellular organism those mechanical conditions consequent on cell-division, formation of a segmentation cavity, invagination, and so forth, are not required to be fulfilled. Surface tension and other extrinsic factors of development of Metazoa have not been shown to exercise a controlling influence in the regeneration of such an organism.² It occurred to me, therefore, that the *ciliate Infusoria*

¹ Morgan's explanation, that the failure to develop is due to inability to produce a sufficient number of cells for the next ontogenetic stage, will come under the first or second of these alternatives, according to the general point of view.

² Of course it is possible for any one to maintain that extrinsic forces do control the regeneration. But the burden of proof rests upon the maker of such an assumption.

offer conditions for decision between these two alternatives. If it should be shown that a nucleated portion of the body below a certain minimal size is incapable of regeneration, the first alternative would receive support. If, on the other hand, the smallest nucleated fragments of the body are capable of regeneration with restoration of the normal form, the first hypothesis would fall, and the second tend to be established.

My material consisted of two species of *Stentor*, viz.: *S. polymorphus* and *S. coeruleus*. The former species occurred in immense profusion on decaying leaves of the water-lily in a small pond near Ann Arbor, the latter appeared in considerable numbers in a small aquarium which had stood in the laboratory for six or seven weeks, and contained gatherings from a swamp. This species is more favorable for experimental work than the former, because the protoplasm is transparent, enabling one to see the nucleus readily in the living animal. In *S. polymorphus* the body is rendered almost perfectly opaque by the presence of immense numbers of symbiotic unicellular Algae, the so-called zoochlorellae, which either hide the nucleus completely from view, or permit mere momentary glimpses of it. On account of the ease of procuring any desired supply of *S. polymorphus* my work was done chiefly on this form; but the results were checked on *S. coeruleus*, and were practically the same for both species.

To reach the desired result it was necessary to find or devise a method by which nucleated fragments of every possible size, beginning with a portion not much larger than a single node of the nucleus, could be produced in large numbers; for reliable quantitative results can be reached only by observation of a large number of cases of regeneration. For this purpose I tried the method of *shaking* which has yielded such admirable results with the animal ovum in the hands of Wilson, Driesch, Morgan, and others, and found it to succeed to perfection. If a number of *Stentors* are put in a small vial about one-third filled with water and shaken quite violently from five to twenty times (*S. coeruleus* requires to be shaken only about five times; *S. polymorphus* ten to twenty times), and then examined under a low power of the microscope, one sees that

the animals have been broken into numerous fragments of every possible size and shape. In the field of the microscope there are present at the same time naked nodes of the nucleus either single or united in groups of two or three, and parts of the body, both nucleated and unnucleated, ranging in size through every possible gradation from 25μ in diameter to about 200μ . Most of the latter are being driven hither and thither by the action of the cilia with which they are covered; and many of them are of the most bizarre and curious shapes: T-shaped, Y-shaped, or provided with other arm-like processes, or of forms impossible to describe; but most of them are of more regular form, triangular, quadrilateral, oval, and spherical.

The moniliform character of the nucleus in these species of *Stentor* insures that a large proportion even of the smallest pieces receive at least some part of the nucleus. In order to satisfy myself that such is the case, I killed and stained the whole of one lot of *S. polymorphus*, which had been shaken as described, about fifteen minutes after the operation. The stained material was then mounted in balsam and measurements were made of the smallest nucleated pieces. Some of the measurements were as follows:

1. Naked nodes of nucleus, spherical or oval; $20-25\mu$.
2. A spherical piece 31μ in diameter containing a single node of the nucleus. Nucleus excentric. Protoplasm a thin cortex.
3. A spherical piece 37μ in diameter; contained a single node of the nucleus.
4. A spherical piece 37μ in diameter; contained two nodes of the nucleus.
5. A spherical piece 40μ in diameter; contained six nodes of the nucleus.
6. A spherical piece 50μ in diameter; contained one node of the nucleus.
7. A spherical piece 50μ in diameter; contained two nodes of the nucleus.
8. A spherical piece 50μ in diameter; contained four nodes of the nucleus.

9. A spherical piece 66μ in diameter ; contained a single elongated node of the nucleus.

10. A spherical piece 69μ in diameter; contained seven nodes of the nucleus.

Other similar pieces containing one or more nodes of the nucleus were seen; some were of course not spherical, but I have given the spherical pieces as easier to compute the volume. Of larger nucleated pieces there was no lack ; they were very numerous, as one would expect. There were, of course, numerous unnucleated masses of protoplasm of various sizes, but few large pieces. In fact, the majority of the pieces below 100μ in diameter were unnucleated; but the above list shows that a good many of such small pieces contained one or more nodes of the nucleus. I did not attempt to ascertain what was the proportion of nucleated to unnucleated pieces of such small size ; but it must have been quite large, perhaps one to ten or even more.

One further remark as to my methods. When any doubt existed in my mind as to the presence of parts of the nucleus in examples noted, the specimen was killed and stained, generally in Schneider's aceto-carmin, and the actual condition of the nucleus thus determined with certainty. This of course involved the sacrifice of a great deal of material.

In consequence of the often curious and asymmetrical shapes of the pieces produced by shaking, I expected to obtain valuable results on the teratogeny of the Stentors for comparison with the results of Balbiani and Johnson, but I have been almost completely disappointed in this respect. When regeneration of a piece takes place at all, it almost always happens that a single more or less perfect animal of typical form results.

RESULTS.

In this paper I shall speak only of results obtained on the smallest parts capable of regeneration, leaving other questions suggested by the experiments for future consideration. From numerous experiments, involving many hundreds of *S. polymorphus*, it was found that the smallest parts capable of

regeneration possess the volume of a sphere of about 80μ diameter. A lesser number of experiments on a smaller number of *S. coeruleus* yielded results almost identical. In the following list I give measurements of some of the smallest Stentors found. After measuring in a more or less expanded condition the animals were made to contract, when they assumed almost the form of a sphere; the diameter of the sphere was then measured, and this measurement was used for comparison.

1. *Stentor coeruleus*. 45½ hours after shaking. Regeneration was complete or nearly so. I could see the adoral spiral sinking into the oesophagus, the mouth, and contractile vacuole. When expanded the form was quite typical. There were two *separated nodes* of the nucleus present.

Measurements. None were made of the expanded animal. Diameter of contracted animal (spherical) 90μ .

2. *S. polymorphus*. 67 hours after shaking. Regeneration complete.

Measurements. a. Expanded, 257μ in length; 80μ across frontal field. b. Diameter of contracted animal (spherical) 80μ .

3. *S. polymorphus*. 70 hours after shaking. Regeneration complete.

Measurements. a. Expanded, 257μ in length; 84μ across frontal field. b. Diameter of contracted specimen (spherical) 87μ .

4. *S. polymorphus*. 96 hours after shaking. Regeneration complete. The animal was sluggish and did not expand well.

Measurement. Diameter of contracted specimen 75μ .¹

I have measurements of a number of Stentors of slightly larger size than the ones given; but the smallest Stentors were very scarce. By far the greater number of nucleated parts, which possessed a spherical diameter of less than 100μ , were incapable of regeneration, or at any rate did not regenerate. However, but a single example is sufficient to show that a portion of the volume of the example in question is capable of regeneration.

¹ My note-book expresses a little doubt about this specimen, but it was certainly under 80μ .

The volume of the smallest Stentor found was thus equal to a sphere of somewhat less than 80μ in diameter. Not one of the hundreds of smaller nucleated parts regenerated, though I found one part, 71μ in diameter in spherical form, which had assumed a fairly typical form of semi-contraction and possessed a single bead of the nucleus; anterior and posterior ends (or foot) were thus recognizable, but there was neither oesophagus nor adoral membranellae present. Even if we admit this as regenerated, which I do not, it does not essentially alter the final result.

My conclusion is, therefore, that nucleated parts of *Stentor polymorphus* of less volume than a sphere of 80μ (approximately) in diameter are incapable of regeneration; nucleated parts of greater volume are capable under favorable conditions of complete regeneration.

The main results hitherto reached on the merotomy of the Protozoa can be summarized as follows:

1. Cytoplasm without nucleus is incapable of regeneration (Nussbaum, Gruber, Verworn, Balbiani, and others). This I can confirm. (Verworn has shown that the isolated central capsule of *Thalassicola nucleata* from which the nucleus has been removed is capable of partial regeneration, but it soon goes to pieces. Gruber has shown that if a Stentor in process of fission be transversely divided so that the posterior part receives no nucleus, this part is nevertheless able to regenerate.)

2. Nucleus without cytoplasm is incapable of regeneration. (Verworn, Balbiani.) This also I can confirm.

3. Portions of the body consisting of nucleus and cytoplasm are capable of regeneration. *To this I must add: provided that the amount of cytoplasm exceed a certain minimal volume* (which in the case of Stentor at any rate is quite considerable).

This amounts to a demonstration of Verworn's view that regeneration in the Protozoa is due to the reciprocal interaction of nucleus and cytoplasm. Organization resides in the cytoplasm as well as in the nucleus. How otherwise are we to explain the fact that a difference in the amount of cytoplasm alone (equivalent to the difference in volume of two spheres of 80 and 70

micromillimeters respectively) determines the occurrence of regeneration?

As regards the bearing of the results on the limits of divisibility of living matter: we are not concerned here with the question of the ultimate constitution of protoplasm, its composition of any ultimate vital elements whatsoever, but merely with the question propounded by Loeb, "What is the order of magnitude of the smallest particle that can show all the phenomena of life?" In the case of the animal ovum as already noted this is about one-fourth of its volume, if we include development as one of the phenomena of life. Certainly development includes all the phenomena of life. In the case of Stentor the volume is relatively considerably less as the following calculation will show:

The volume of the smallest perfect *Stentor polymorphus*, which I was able to produce, was equal to that of a sphere of about 80μ diameter; the average volume of the Stentors used in the experiments was equal to that of a sphere of about 230μ , as I determined from a series of measurements of animals killed in a weak killing-fluid, and thus completely contracted. That is, the ratio of the diameters of the smallest and the average Stentor is about 1:3; or the ratio of volume to volume is about 1:27. That is to say that the smallest Stentor which can be produced is about one twenty-seventh of the volume of the average Stentor.¹ This number is of course a mere approximation, but it certainly will not be made any greater by subsequent investigation, though it may be lessened somewhat.

¹ In the case of *S. coerules* the figures are different: the smallest measurement which I have of this species is 90μ ; the average is 280μ ; thus the ratio of the smallest to the average is about 1:3 in terms of the diameter, or 1:27 in terms of volume. I believe, however, that it would be possible to produce a smaller *S. coerules* by working over a larger amount of material. I do not think that there is much difference in the *absolute* size of the smallest Stentors which can be produced, whether one uses the largest or smallest normal specimens. If e.g. the average size of a lot of large Stentors were 320μ , the smallest specimen which could be produced would still be 80μ . The ratio of volumes would then be 1:64. Of course this does not necessarily mean that 64 Stentors could theoretically be produced at one time from a single one, for I doubt that the nucleus could undergo that amount of division.

In any case this relation forms a striking contrast to that found in the development of the animal ovum, where a portion of less volume than one-fourth that of the ovum does not develop into an embryo (see postscript). It has been very generally found that a portion (of the two- or four-cell stage) equal to one-eighth the volume of the ovum never develops farther than the gastrula stage.

In the case of the animal ovum, again, parts slightly smaller than the minimum necessary for the complete development may undergo partial development. In *Stentor* we have a parallel phenomenon: parts of less than 80μ spherical diameter may undergo partial regeneration, but are unable to complete it.

It seems to me that neither increased surface tension due to diminution of surface area, nor yet any other external factor, is responsible for this failure of small pieces of *Stentor* to regenerate. The cause lies within; and I do not believe that it is to be sought in an insufficient production of energy. For such small pieces may live for days, constantly producing and expending energy in the ordinary processes of metabolism. I am forced, therefore, to the conclusion that the organization of these parts is in some way deficient. *There is probably for each species of animals a minimal mass of definite size consisting of nucleus and cytoplasm within which the organization of the species can just find its latent expression. This is the minimal organization mass.*

In the case of the Protozoa the size of this minimal mass is that of the smallest part capable of complete regeneration. But I do not believe that in the Metazoa the minimal organization mass is that of the smallest part of the ovum capable of developing into a normal embryo; for undoubtedly the influence of external factors is of the greatest importance here. I would conceive then that in the Metazoa this hypothetical minimal organization mass is smaller than any part yet observed to develop into a normal embryo. Still, from my results on *Stentor*, I believe that it is of such a size as to be easily visible under a low power of the microscope.

POSTSCRIPT. — After sending the above article to the editor I had access to Boveri's recent paper entitled "Ueber die Befruchtungs- und Entwicklungsfähigkeit kernloser Seeigeleier und über die Möglichkeit ihrer Bastardirung," published in Bd. II, Heft 3, of Roux's *Archiv für Entwicklungsmechanik der Organismen*, Oct. 22, 1895. Boveri states that the smallest dwarf larva which he obtained came from a fragment which could not have measured more than $\frac{1}{20}$ the volume of the intact ovum "bei ungünstiger Rechnung." His conclusion is: "Das Fragment des Seeigeleies bis herab zu einer Grösse von $\frac{1}{20}$ des ursprünglichen Eivolumens besitzt die formative Wertigkeit des ganzen Eies." This is in marked contrast to the results of the other authors quoted, none of whom have found a figure less than $\frac{1}{4}$. The difference may be due in part to the fact that Boveri shook the ova before fertilization, while the other experimenters performed this or an analogous operation after fertilization; although this does not seem very probable. If the exact proportion of the minimal organization mass to the whole ovum be a matter of any importance, very great care in the estimation of the volumes of dwarf larvae would seem to be necessary, taking into account the differences in thickness of the layers in dwarf and normal larvae, and also the relatively slow increase in volume of the former.

The figure which I have found for Stentor is but little lower than that of Boveri for the animal ovum, and this approximation suggests interesting comparisons.

ORGANIC VARIATION AS A CRITERION OF DEVELOPMENT.

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INTRODUCTORY.

THE object of the present paper is, firstly, to give a tentative explanation for the origin of variation, deducible from the law of the concomitance of variation with continuing development. The attempt will then be made to show that variation is caused indirectly by change of environment, and directly by the disturbance of correlation of the organs, resulting from the change of environment. And secondly, the attempt will be made to determine whether, in a given organism, the amount (or degree) of variation, and the manner of its occurrence, can furnish us with criteria for judging its lines of development. The only postulates necessary for the treatment of the problem of variation from this point of view are: (1) the concomitance of variation with continuing development, (2) the correlation of the organs of an organism, as necessary for its existence, and (3) the influence exerted upon the organism by its environment, necessitating a degree of adaptation to its environment.

It is not my intention to review the many theories already advanced to explain the nature and origin of variation, which would be a task too extensive for the scope of my present article. There are two well-known theories, each of which has latterly been more or less modified in regard to the origin of variation : the first teaches that variation is caused more or less directly by the environment ; while, according to the second, variation is the result of an inherent tendency on the part of the organism to vary. Now, in regard to the last-named theory, it may be said with truth that, though much may be explained on the assumption of "inherent tendencies," there is no empirical proof of the existence of such tendencies ; and further, variation is not explained by the assumption of an inherent tendency to vary, until the origin and nature of the inherent tendency itself be explained. Reference may also be made to the theory of Weismann, that variation has its origin in conjugation. My own theory, as will be seen in the following pages, inclines somewhat to the doctrine of the origin of variation as caused by the influence of the environment, but is a new departure from the Lamarckian theory, inasmuch as I consider variation to be possible only under a temporary state of independence of the several organs, when their complete correlation has been disturbed by a change in the environment.

The recent admirable work of Bateson¹ has shown clearly the importance of a careful comparative study of the phenomena of variation, for the understanding of the problems of morphology. In his book he has treated principally of the phenomena of variation in their relation to certain laws of bilateral and radial symmetry ; but the problem of the origin of variation he dismisses by stating that "Inquiry into the causes of variation is as yet, in my judgment, premature" (p. 78). My reason for attempting the solution of a problem so intricate and difficult in its nature is the need of approaching the question from a new point of view ; and in this attempted solution I have endeavored to keep within the line of facts as much as possible, and to avoid making unnecessary theoretical assumptions.

¹ Wm. Bateson : *Materials for the Study of Variation, treated with especial regard to Discontinuity in the Origin of Species*. London, 1894.

Although the doctrine of Natural Selection will be but little mentioned in the following pages, it is nevertheless advisable to give a clear definition of it. Darwin states ("Origin of Species," new American edition from sixth English edition, 1886, p. 63) : "This preservation of favorable individual differences and variations, and the destruction of those which are injurious, I have called Natural Selection, or the Survival of the Fittest." If we take this definition, and eliminate from it the sentence, "and the destruction of those which are injurious," we have before us perhaps a true explanation of the origin of species. But the elimination of this passage seems necessary, since it is a debatable question to what extent the "destruction" of the unfavorable variations can proceed. Darwin himself discussed the preservation and destruction of variations, and left untouched the problem of their origin ; but in the "Origin of Species" he makes two statements which are of interest here : "It seems clear that organic beings must be exposed during several generations to new conditions to cause any great amount of variation ; and that, when the organization has once begun to vary, it generally continues varying for many generations" (p. 5). "Unintentionally he [man] exposes organic beings to new and changing conditions of life, and variability ensues ; but similar changes of conditions might and do occur under nature" (p. 62). From these two quotations we may conclude that Darwin considered variation to have its origin in change of environment, — in which view he probably followed Lamarck.

I. DEFINITION OF VARIATION ; CORRELATION OF ORGANS ; PROGRESSIVE AND REGRESSIVE DEVELOPMENT.

Bateson applies the following definition to variation (*l.c.*, p. 3) : "To this phenomenon, namely, the occurrence of differences between the structure, the instincts, or other elements which compose the mechanism of the offspring, and those which were proper to the parent, the name *variation* has been given." But since cases are known in which wholly normal offspring have descended from parents which were not normal in all respects, we cannot consider the offspring in such cases to present variations

from the specific type, even though they may differ from their parents ; so that it is advisable to seek a more general definition. Accordingly, organic variation may be defined as growth above or below (*i.e.* beyond) a given norm ; and organic variability, the power of the individual organism to produce such variation. Thus we can only then speak of variation when at a particular point in the ontogeny the growth of a given organ in one individual is greater or less than the normal growth at that stage. It remains necessary, however, to apply distinctive definitions to the terms "normal" and "abnormal" ; and, although it is not possible to give strictly distinctive definitions to such relative ideas, it is, however, generally understood that such characters are *normal* as are presented by the greater part of the individuals of a given species, and such *abnormal* as are presented by a much smaller percentage of individuals. Though no really distinctive definitions can be given, the relative meanings of normal and abnormal are sufficiently understood, which is all that our definition of variation demands. And even in cases of a more or less perfect intergradation of the degrees of development of a given organ, in a large number of individuals of a species, it seems to be always possible to show that the limits of variation of the large majority of individuals lie within a certain circumscribed compass ; so that here normal and abnormal degrees of variation may be distinguished.¹

By the expression "Correlation of the Organs," is understood the state of mutual dependence of the organs, after their division of labor has been brought about by the process of evolution ; each has its own particular function to perform, but the fulfilment of this function is not sufficient for its existence, since rather it would be unable to perform its own function without the aid it derives from the other organs. But further,

¹ The term *variety* is often used ambiguously, as synonymous with variation, or as equivalent to the idea subspecies (geographical race). In the strict sense, however, the term variety is applicable only to the whole individual, and not to a single organ of it, and therefore is not equivalent to variation, which is any growth above or below the normal. A variety is then, *sensu stricto*, synonymous with the term subspecies ; but in order to avoid any possible ambiguity, which has risen from the wrong use of the word variety, I shall in the following pages avoid adopting it, and shall use instead *subspecies*, or *geographical race*.

while this physio- and morphological correlation of the organs aids each organ in the fulfilment of its proper function, it simultaneously acts as a restraint upon the exertions of the vital processes of the particular organ; for the particular organ does not functionate merely for the maintenance of its own existence, but primarily for that of the whole organism, and when it has fulfilled the demand of the whole, its correlation with the other organs would cause a temporary cessation of its activity. Thus the correlation of the organs exerts a restraint, — acts as a regulator, upon the amount which each shall perform. And when the correlation is perfect, we must assume that each organ can normally perform a certain fixed amount, and no more nor no less. Since the performance of a physiological function results in morphological change, showing the direct correlation of the function and structure of an organ, the result follows that, if the amount of physiological action performed by an organ is determined by the correlation of the organs, the amount of morphological change must be determined also by the correlation of the organs. This fact is important for the establishment of the deduction, which will find its treatment further on, that variation can appear only when the complete correlation of the organs has been disturbed. And since the degree of perfection of the division of labor between the several organs is proportional to the amount of differentiation of the organism, it is correct to conclude, that the completeness of the correlation of the organs stands in a direct proportion to the degree of differentiation of the organism, — the higher the organism the more perfect is the correlation of its organs; and *vice versa*, the lower the organism is structurally, the less intimate is this correlation, *i.e.* the more independent the several organs are.

It will be well here to analyze and compare briefly the ideas, *progressive* and *regressive development*. Either process may modify a given organ in regard to its structure (chemical and morphological), its size, position, and, in meristically arranged organs, its number. Progressive development tends to complicate or further differentiate its chemical and morphological structure, to change its position and dimensions, and (subject to certain limitations) to increase its number in a meristic

series; while regressive development (degeneration, *Rückbildung*) tends to simplify the structure, to change the position and dimensions, and (subject to certain limitations) to decrease its number in a meristic series. Since either process may produce a change of position of the organ involved, such a change furnishes no criterion whereby to judge the kind of development, until it can be determined whether the direction or amount of change of position differs according as the mode of development is progressive or regressive. And whether change of the dimensions gives us a criterion of the kind of development is also doubtful; although, since it is the general rule that increasing complexity of structure is usually accompanied by actual increase in size, it might be concluded that an increase in size of an organ denotes frequently the action of progressive development. But since it is only a general rule, and by no means a law without exceptions, that increase in size goes hand in hand with increasing structural complexity, it would be safer to eliminate change of dimension from our criteria for distinguishing between the two modes of organic development.

We find, however, a certain criterion for estimating the kind of development, in change of structure; for an increasing complexity of structure is distinctive of progressive development, while, on the other hand, a decreasing complexity is the sign of regressive development. Further, an increase in the number of meristically (segmentally or metamerically) arranged organs is a criterion of progressive development, as a decrease in the number of similar organs is of regressive development, — provided that, in each case, no structural changes are simultaneously taking place. This standpoint will hardly be disputed, for we consider an organism *A* to be morphologically higher than an organism *B*, when *A* possesses a larger number of organs in a given meristic series than does *B*, even though these organs are otherwise structurally equivalent in *A* and *B*.

But it is necessary to consider the case, when a progressive meristic development is acting together with a regressive structural development, or *vice versa*: as *e.g.* in the carpus of the Ichthyosauria, where the phalanges are meristically pro-

gressive, but regressive in regard to specialization, in comparison with their ancestral forms. The question before us is, then: When in an organ there is at work a progressive structural development, simultaneous with a regressive meristic development, is the organ to be regarded on the whole as progressive or regressive? The answer to this is to be gained by determining whether structural complexity is of greater or of less morphological importance than is meristic change. Now the consensus of opinion among biological investigators would show that structural complexity (both chemical and histological) is of much greater morphological importance than is mere meristic development, — this assumption being, indeed, a necessary preliminary in any attempt to homologize different organisms. For to pick out an example at random, who would venture the opinion that *Branchipus* occupies a higher morphological position than *Astacus*, simply on the ground that it possesses more numerous extremities; and would not rather conclude that *Astacus* is the higher organism, because its extremities are structurally more differentiated? Therefore, if structural modification is of greater morphological importance than numerical (meristic) modification, then when progressive structural development is accompanied by regressive numerical development, the organ as a whole is to be regarded as developing progressively; and conversely, when regressive structural development is simultaneous with progressive numerical development, the course of development of the organ is to be considered regressive.

It still remains to accentuate an apparent law, which is generally recognized, with reference to this frequent concomitance of numerical and structural development. Apparently a progressive structural development frequently causes a regressive numerical development, as we find when, by the coalescence of previously separate meristic organs (*i.e.* through their regressive numerical development), a compound organ is produced, which is higher morphologically than was any one of the previously separate organs. However, a numerical reduction of the units of a meristic series can proceed, and perhaps does so more usually, without

coalescence ; and such a regressive numerical development is also usually associated with a progressive structural development of those units of the meristic series which are retained. Examples of such cases may be found in abundance, and it is sufficient here to refer to the lateral line of sense-organs in the Vertebrates, where a progressive development of certain of the individual organs is concomitant with a reduction in the number of the units comprising the series. And with a view to many analogical cases, which it is unnecessary to give in detail here, since any zoölogist may recall many to mind, the law will be found to be of general application, that progressive structural development is furthered by regressive numerical development, since in this way greater centralization of the forces of growth would ensue. And although regressive structural is sometimes concomitant with regressive numerical development, as exemplified in the case of certain parapodia of sedentary Annelids, I recall no case of the concomitance of progressive structural and progressive numerical development ; but I would not imply by this that such a concomitance cannot or does not occur, but rather that such a concomitance is of so infrequent occurrence as not to render invalid the general law just mentioned. My reason for accentuating this law of the usual concomitance of progressive structural with regressive numerical development is in order (1) to characterize concisely a relation, which, although already recognized, has not yet been awarded much attention from zoölogists ; and (2) to emphasize a point which may serve as a criterion of progressive development.

By the term development is meant here any organic process of change acting in the organism ; when such a change tends to further complicate the structure, it has been termed progressive development (evolution, *Entwicklung*) ; when it tends to simplify the structure, regressive development (degeneration, *Rückbildung*). Speaking generally, development leads towards (1) the production of new species, or (2) towards the extinction of already present species ; obviously, development cannot be conceived as holding a species stable (*i.e.* unchanging), since development always implies an organic change.

After these preliminary explanations, we may next consider the phenomena of continuing development.

II. CONTINUING ORGANIC DEVELOPMENT IS ALWAYS ACCOMPANIED BY VARIABILITY.

Although this postulate may seem at first sight to be a mere enunciation of a well-known biological axiom, and a *sine qua non* of the theory of development, it is nevertheless of great importance for arriving at a true conception of the nature of variation; and although evolutionists in general will grant with Darwin that for the action of Natural Selection the occurrence of variations is necessary, yet to my knowledge no one has particularly accentuated the fact of this actual concomitance of variations with continuing development. Indeed, most biologists have accepted this fact, without a critical inquiry into its fundamental importance. In my last paper¹ I laid particular stress upon this point, by saying (p. 483): "Now I consider this variability in the number of the eyes of the freshwater forms to be explained by the general law, that all organs (and *propter hoc* all organisms) which are undergoing progressive or regressive development tend to be variable." In the present paper I hope to substantiate the validity of this "general law" by data from another source.

A. *Certain Criteria of Continuing Development.*

In order to prove the assumption that continuing progressive and regressive development is always accompanied by variability, it is necessary to produce a series of facts, showing that organs (or organisms) which are undoubtedly in a state of continuing development always evince variability. But, although examples of variation may be found in abundance, it is obviously difficult to prove conclusively that a given organ (or organism) is at a given time influenced by a continuing process of development. Accordingly, for each example to be cited, we

¹ "The Derivation of the Freshwater and Land Nemertean, and Allied Questions." JOURNAL OF MORPHOLOGY, XI, 2, 1895.

must demonstrate satisfactorily that it is undergoing a process of development.

The question to be solved is then, first of all: What are our criteria of continuing development? Progressive and regressive development having been sufficiently characterized, it remains necessary to produce criteria, whereby we can determine whether a given organ (or organism) is at a particular time dominated by a process of development, or whether the organ (or organism) is not being influenced by a particular developing agency, either progressive or regressive. We may now consider briefly three reliable criteria of continuing development, namely, (1) domestication, (2) the presence of geographical races (subspecies), and (3) migration; no doubt other criteria may be found, but these three are sufficient for our present purposes.

(1) *Domestication* may be taken as a criterion of continuing development, since all organisms in a state of domestication are being more or less continuously selected by man, with a view to their adaptability for certain uses. The development induced by human agency is also very energetic, since man's uses for domesticated animals and plants are manifold, and since he frequently introduces changes in their environment. And further, as we know in many cases that the length of time necessary for the production of a new "breed" has been comparatively short, we must conclude that not only was the action of the development continuous, but also that it must needs have been very energetic.

(2) *The presence of geographical races* may also be considered a criterion of continuing development. A species is said to present geographical races or subspecies when in different portions of its breeding area particular forms occur, differing mainly in color and dimensions, but which are all connected together by a more or less perfect series of intergradations, and all of which may breed together fertilely. Any one at all conversant with the geographical distribution of animals or plants knows how frequently wide-ranging species are differentiated into a number of geographical races, and that the number of such races stands usually in a direct ratio to the extent, or diversification, of the

range of the species. Now if the Darwinian doctrine of evolution be true, all such races have descended from one ancestral form ; but I think that we may go still further, and postulate that wherever one geographical race grades insensibly into another, there the agency of development must be still continuing. For supposing *A*, *B*, *C* to be three intergrading geographical races inhabiting contiguous areas *a*, *b*, *c*. In area *a*, together with the individuals of race *A*, will always occur some individuals of races *B* and *C*, which have migrated from *b* and *c* into *a*. Now these individuals, which have migrated from *b* and *c* into *a*, must adapt themselves to the new environment of the area *a*, if they would compete successfully with the individuals of race *A* ; and thus a continuous development of a considerable number of the individuals of the species must proceed, tending to produce favorable adaptations in the struggle for existence, — this struggle being probably keenest where the areas *a*, *b*, and *c* overlap. It is still a bone of contention between systematic ornithologists whether individuals of a race *B* are ever found in the area proper to a race *A*, or *vice versa* ; but the cases where this is so, as *e.g.* *Dendroica palmarum* and its variety *hypochrysea*, are so numerous as to warrant the conclusion that, wherever the geographical lines of demarcation between the respective breeding areas are not strongly marked, there must be a considerable interchange of wandering individuals. And it seems to be always the rule, that the indefinitely broad area of demarcation is peopled promiscuously by individuals of the contiguous races. Only when the lines of demarcation are formed by high mountain ranges, deserts, or great water expanses would there be little or no interchange of individuals ; but when the boundaries of the areas of the several forms are so comparatively impassable, the various forms usually do not perfectly intergrade, and hence are to be classed rather as separate species than as races of one species. Therefore it is correct to conclude that in a widely ranging species, split up into a number of intergrading geographical races, a continuous agency of development is at work, leading to the readaptation of the migrating individuals to new environments.

Darwin ("Origin of Species") has ably argued the point

that where the adjoining areas of geographical races overlap, the struggle for existence would be keenest, so that the individuals occupying this intermediate area would in time become extinct. But he has overlooked the fact that until such extermination has been brought about, *i.e.* as long as the races continue to intergrade, the intermediate area would continue to be the vortex of development, and the sharp struggle there would itself instigate the wandering of individuals into the adjoining areas, where again they must adapt themselves to new environments. Now, when in any species the individuals occupying the areas transitional between those proper to the several races have become exterminated, the races must cease to intergrade, fewer individuals will continue to wander into other areas, and, the struggle for existence becoming less sharp, the main factor in the process of development would disappear. But, as we have shown above, when the races cease to intergrade, and become more distinctly pronounced, they can *sensu stricto* be no longer termed races, but rather distinct species. Accordingly, the presence of geographical races being correctly considered a criterion of continuing development, we should expect our data to show, as indeed they do, that, other factors being equal, species with geographical subspecies evince a greater amount of variation than do species which present no geographical races.

It is noteworthy that extensive periodical migrations act as a restraint upon the production of geographical races. And I account for this fact¹ by assuming that the migratory species, being influenced in winter by an environment to some extent different from that which it experiences in summer, must be equally adapted to both environments (*equally*, at least, if it remains under the influence of both environments for the same length of time), and hence, the winter environment exerting a restraint upon the production of adaptations suited to the summer environment alone, such a migratory species would not be capable of producing geographical races to the same

¹ In a paper which has not yet appeared, but which will be published in the *American Naturalist* for June, 1896, dealing with migration as a check upon geographical variation in birds.

degree, as would a non-migratory species with an equally extensive breeding area.

(3) *Extensive migration* may be taken as another criterion of continuing development. (By the term *extensive* migration, I mean, as will be explained later, a regular periodic migration through a considerable distance, — 30° lat. or more.) For, as was shown in the preceding paragraph, a migratory species in wandering from its summer to its winter home, or *vice versa*, is brought into contact with a different environment, necessitating a certain amount of readaptation ; therefore, there must be at work a more or less continuous process of development, leading towards readaptations. Thus, to use a well-known example, a man accustomed to spend his annual holiday abroad, on arriving at his destination experiences the lassitude preparatory to his becoming acclimated, and experiences the same physical sensations on his return. Accordingly our data should demonstrate that species which undertake periodic migrations through long distances should evince a greater amount of variation than do stationary species, other factors being equal in amount.

Other criteria of continuing development might be mentioned, but as the three already given are well founded and sufficient for the furtherance of our deductions, we shall deal but briefly with a fourth. If Wallace's theory ("Darwinism") be true, that secondary sexual characters are most accentuated in those species where the sexual impulses are strongest, so that the sexual impulse may be considered as an important if not sole agent in their production, then we might consider the presence of strongly marked secondary sexual characters as a criterion of continuing development, by regarding the sexual impulse itself as a more or less continuing impulse to development. In other words, the degree of development of secondary sexual characters would stand in direct proportion to the continuousness and energy of the sexual impulse. And since secondary sexual characters are often different in otherwise closely allied species, being as a rule the least reliable (morphologically speaking) of specific differences, they must be regarded as of comparatively recent origin in each species, and thus be considered characters prob-

ably still under the agency of a process of development. But though this reasoning may be plausible, it is based upon Wallace's assumption that the production of such characters is due to the agency of the sexual impulse ; and rather than bind myself to such a theory, I would leave the case still disputable, whether or no the presence of noticeable secondary sexual characters should be taken as a criterion of continuing development.

B. *Data.*

It now remains to produce data in support of the thesis that individual variation is always concomitant with continuing development ; and to do this, it must be demonstrated successively that (1) variation is always predominant in those domestic animals which have been most carefully selected by man ; (2) in such species as are divisible into geographical races ; and (3) in those species which undertake extensive periodical migrations.

Variation in domesticated animals is very marked, and especially so in those forms which have been most carefully selected by man. It is of interest to compare the diversity of breeds of the dog with the fewer breeds of the cat ; the former is of greater practical use than the latter, and man has subjected it consequently to a greater diversity of conditions of life. Whether a greater amount of individual variation is evinced by the domesticated animals than by their allies in a state of nature, cannot as yet be answered with certainty, since, as Bateson (*l.c.*) observes, the phenomena of variation in the wild forms are not known to the same extent. Cope ("Origin of the Fittest") mentions the peacock and the Guinea fowl as forms which have not been rendered variable by domestication ; but these two may be classed as the wildest, and least carefully bred by human selection, of any of the domesticated birds. However, without going further into the much-discussed question of variation under domestication, the fact is sufficient for us that animals show considerable individual variation under domestication, and that the amount of variation is greatest in those species which have been most influenced by human selection ; and we

have found domestication to cause a more or less continuous development.

In order to test the correctness of the assumption that individual variation is most marked (1) in those species which possess geographical races, and (2) in those species which undertake extensive migrations, I have examined nearly all the species of North American birds with reference to individual variation in some or all of the following dimensions : culmen of the bill, wing (from carpal joint to tip of longest primary), tarsus (so-called, but really tarso-metatarsus), whole length (from tip of bill to tip of tail), and tail (from the pygostyle to tip of the longest rectrix). It was my original intention to personally undertake all the measurements, and with that object in view I commenced a series of detailed measurements upon the birdskins in the collection of the Academy of Natural Sciences of Philadelphia. Unfortunately for me, however, this collection did not offer large enough series of individuals of all the species desired, and not having the opportunity nor time to study other large collections, — namely those at Cambridge, New York, and Washington, — I was obliged to desist from further personal examinations. In lieu, then, of such direct examination, I have taken Robert Ridgway's excellent "*Manual of North American Birds, 1887*" (first ed.) as my authority for the extremes of individual variation, in regard to the dimensions specified, of the North American species of birds. And here I would express my hearty gratitude to Professor Ridgway for his liberality and generosity in allowing me to make use of his valuable data. In speaking of the measurements given in his work, Ridgway states (p. ix) : "Whenever practicable, they have been taken from large series of specimens, and the extremes given as well as the average. . . . In the case of closely allied forms, or where distinctive characters are largely a matter of dimensions or the proportionate measurements of different parts, care has been taken to measure, whenever possible, an equal number of specimens of the several forms to be compared ; and specimens in abraded or otherwise imperfect plumage, as well as young birds, have been excluded. When there is any marked sexual difference in size, the number of males and females measured

of allied forms has also been made as nearly equal as possible." The degree of individual variation in regard to the dimensions, according to Ridgway, is therefore based (for most of the species) on large series of specimens of adult individuals, in unworn plumage; and as this distinguished ornithologist's work is regarded as a standard by taxonomists, the accuracy of his measurements cannot be questioned. And as such a large series of data is the result of years of painstaking work, I may be pardoned for not attempting such a labor in the limited time at my disposal. I have taken these measurements as given by Ridgway, with necessarily the exclusion of such extremes of variation as were based upon a very small number of individuals, and have computed the percentage of variation for each given dimension, expressing the difference between the extremes of variation as a percentage of the minor term of variation. In this way I have deduced the percentage of variation in the dimensions of the larger part of the species and subspecies of North American birds (together with those of a considerable number of exotic species, of casual or possible occurrence within our boundaries); or, altogether, the species and subspecies of fifty-six families, the only omissions being the following small families: *Trogonidæ*, *Alcedinidæ*, *Momotidæ*, *Cotingidæ*, *Hirundinidæ*, *Ampelidæ*, *Laniidæ*, *Coerebidæ*, *Motacillidæ*, *Cinclidæ*, *Certhiidæ*, *Sylviidæ*. These latter families have been omitted, because their respective scarcity of species would hardly warrant comparisons. Accordingly, using the measurements given by Ridgway as my basis, I have computed the percentage of individual variation for one or more of the five dimensions specified, for the greater number (approximately 600 or more) of species and subspecies of North American birds. It is unnecessary to reproduce in this paper these measurements for all the families, which would only result in the needless occupation of too much valuable space in this JOURNAL; accordingly, for purposes of comparison I will present tables of variation for those families only, in which the diagnostic characters of most of the species are furnished by the measurements, and for which, therefore, the percentages of variation, based upon such necessary accurate measurements, may be considered as accurate as possible.

Together with the degrees of variation, will be given for each species also, as briefly as possible, the range of migration and breeding area. These facts have been extracted principally from Ridgway's work (*l.c.*), and from the recent work of Witmer Stone.¹ Birds with a migration range of 30° lat. north and south, or a corresponding distance east and west across the continent, I have classed as *extensive migrants*; birds with a smaller migration range, as *migrants*; those which do not undertake regular periodic migrations, but occasionally accomplish wanderings of considerable extent, as *irregular migrants*; and finally, those which migrate not at all, or, as the meadow lark and crow, migrate through only short distances, as *residents*. Such a classification according to the range of migration is necessarily an arbitrary one; as is shown *e.g.* by the migration of certain species from high mountain ranges to the adjoining valleys in the winter season, a case which could not be classed as an extensive migration, although each such species meets with a considerable change of environment. This classification has been used merely as a convenience for computing the relation of the amount of variation to the extent of migration of the species. In other words, the extent of migration and the breeding area have been given for each species in order to learn the laws of the amount of individual variation in its relation to the environment.²

¹ Witmer Stone: *The Birds of Eastern Pennsylvania and New Jersey*, etc. Philadelphia, 1894. I would here express my gratitude to my friend Mr. Stone for his valuable aid in helping me to determine the migration and breeding ranges of certain species; and also for the facilities offered me to study the bird collections of the Philadelphia Academy of Natural Sciences.

² The nomenclature adopted here for the species of birds is that employed by the American Ornithologists' Union, with the emendations contained in its supplementary lists. I was unable to consult the second edition of this work, which appeared after this paper had been sent to press.

The following abbreviations will be employed in the tables:

- = variation under 1 %.
- = variation between 1% and 1.5%.
- = variation between 1.5% and 2%.
- = variation of 2% or more.
- R. = resident.
- I.M. = irregular migrant.
- M. = regular migrant.
- E.M. = extensive migrant.
- distr. = district.
- trop. = tropical.
- temp. = temperate.
- G. = gulf.
- c. = centre (or central).
- I. = island.
- int. = interior.
- r. = river.
- vall. = valley.
- st. = state.
- N.E. = New England.
- A. = America.
- C.A. = Central America.
- Miss. = Mississippi.
- B.C. = British Columbia.

n., e., s., w. = north, east, south, west (or their adjectives).

All other abbreviations for states and countries are those commonly employed in the U. S.

TABLE I. VARIATION IN THE RALLIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Rallus crepitans</i> Gmel.	••••	•••	••••	•••		M. Atlantic marshes n. to Long I.
<i>R. c. saturatus</i> Hensh.	•••	*	*	•••		R. coast of La.
<i>R. obsoletus</i> Ridgw.	••	*	*	*		R. coast marshes from L. Cal. to Ore.
<i>R. elegans</i> (Aud.)	•••	•••	••	••		M. freshwater marshes of e. U.S.
<i>R. beldingi</i> Ridgw.	••	••	••	*		R. e. coast of L. Cal.
<i>R. tenuirostris</i> (Lawr.)	••••	*	•••			R. c. & w. Mex.
<i>R. virginianus</i> Linn.	••	*	*	••••		E. M. whole N. A. n. to Hudson B.
<i>Porzana carolina</i> (Linn.)	••••	*	*	••••		E. M. n. U. S. northward.
<i>P. jamaicensis</i> (Gmel.)	••••	••••	*	••••		E. M. s. U. S. n. to Mass. & Ore.
<i>P. noveboracensis</i> (Gmel.)	••••	••••	*	••••		E. M. n. U. S. to Hudson B. w. to Utah.
<i>Ionornis martinica</i> (Linn.)	*	*	••			M. nearly whole trop. & warm temp. A.
<i>Gallinula galeata</i> (Licht.)	*	*	••	••		M. whole trop. & temp. N. A. to B. C.
<i>Fulica atra</i> Linn.	•••	••	*			M. n. Eurasia.
<i>F. americana</i> (Gmel.)	••••	*	••	••••		E. M. n. U. S. to Greenland & Alaska.

TABLE II. VARIATION IN THE FALCONIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Elanoides forficatus</i> (Linn.)	**	**	***	***		M. trop. & warm temp. N. A.
<i>Ictinia plumbea</i> (Gmel.)		***				R. s. Mex. to Paraguay.
<i>Rostrhamus sociabilis</i> (Vieill.)	**	*		**		R. Mex. to Argentine Rep.
<i>Circus hudsonius</i> (Linn.)	+	**		*	**	E. M. whole of N. A.
<i>Accipiter velox</i> (Wils.)	+	***	*	***		E. M. whole of N. A.
<i>A. cooperi</i> (Bonap.)	+	**	**	***		R. temp. N. A. & Mex.
<i>A. atricapillus</i> (Wils.)	+	**	*	**		M. n. e. N. A. n. of U. S.
<i>Parabuteo unicinctus</i> (Temm.)	+	*	*	*		R. S. A.
<i>P. u. harrisi</i> (Aud.)	+	*	*	*		R. C. A. & s. U. S.
<i>Buteo borealis</i> (Gmel.)	+	**	**	***	*	R. e. N. A. w. to Gt. Plains.
<i>B. b. harlani</i> (Aud.)	+	*	**	***	*	R. G. St. & lower Miss. vall.
<i>B. buteo</i> (Linn.)	+	**	*	***	*	R. n. E. Hemisphere.
<i>B. abbreviatus</i> Cab.	+	***	*	**	*	R. n. S. A. to s. Tex. & s. Cal.
<i>B. swainsoni</i> Bonap.	+	**	**	***	*	R. w. N. A. from Alaska to Argentine Rep.
<i>B. brachyurus</i> Vieill.	+	***	***	***	*	R. trop. A. n. to e. Mex.
<i>B. latissimus</i> (Wils.)	+	**	**	***	**	E. M. e. N. A. to Saskatchewan.
<i>B. lineatus</i> (Gmel.)	+	**	*	***	**	M. e. N. A. w. to Gt. Plains.
<i>B. l. elegans</i> (Cass.)	+	**	*	*	***	R. Pacific coast of U. S.
<i>B. albicandatus sennetti</i> Allen	+	*	*	*		R. e. S. A. to s. Tex.
<i>Urubitinga urubitinga</i> (Gmel.)	+	**	*	*		R. Costa Rica to Argentine Rep.
<i>U. ridgwayi</i> Gurney	+	***	*	**		R. Guatemala & s. Mex.
<i>U. anthracina</i> (Licht.)	+	*	**	*		R. trop. A. n. to s. Ariz.
<i>Archibuteo lagopus</i> (Brünn.)	+	*	*	***	**	M. n. E. Hemisphere.
<i>A. ferrugineus</i> (Licht.)	+	*	*	*		M. w. U. S. n. to Saskatchewan.
<i>Aquila chrysaetos</i> (Linn.)	+	*	*	*	***	R. n. portions of N. Hemisphere.
<i>Haliaeetus albicilla</i> (Linn.)	+	**	*	*	**	M. n. portions of E. Hemisphere.
<i>H. leucocephalus</i> (Linn.)	+	***	***	***	***	R. whole of N. A., Kam-schatka.

TABLE II. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Thalassœtus pelagicus</i> (Pall.)	+			•		M. Kamschatka sea-coasts.
<i>Falco islandus</i> Brünn.	•	•				R. circumpolar regions.
<i>F. rusticolus</i> Linn.	••	•	•	•		M. extreme n. N. A. & Eurasia.
<i>F. r. gyrfalco</i> (Linn.)	•••	•	••	••		I. M. n. Europe & arctic A.
<i>F. r. obsoletus</i> (Gmel.)	••	•	••			M. coast of Labrador.
<i>F. mexicanus</i> Schleg.	•	••	•			R. w. U. S. s. to Mex.
<i>F. peregrinus</i> Tunst.	•	•	•••	•		M. Europe and portions of Asia.
<i>F. p. pealei</i> Ridgw.	•••	••	••	••		R. Aleutian Is. & coast of Ore.
<i>F. deiroleucus</i> Temm.	••	•	•			R. trop. A. n. to s. Mex.
<i>F. albigularis</i> (Daud.)	•	•	••			R. trop. A. n. to n. Mex.
<i>F. regulus</i> Pall.	••	•	•			R. Eurasia.
<i>F. columbarius</i> Linn.	•	•	•	••		E. M. N. A. chiefly n. of U. S.
<i>F. c. suckleyi</i> Ridgw.	•	•	••			M. n. Cal. to Sitka.
<i>F. richardsonii</i> Ridgw.	•	•	•			M. int. of N. A. from Col. northward.
<i>F. fusco-cœrulescens</i> Vieill.	••	•	•	••		R. trop. A. n. to s. Tex. & N. M.
<i>F. sparverius</i> Linn.	••	•	••	•		M. whole temp. N. A.
<i>F. dominicensis</i> Gmel.	••	•	••			R. Cuba & Hayti.
<i>Polyborus tharus</i> (Mol.)	•	•	••			R. S. A.
<i>P. cheriway</i> (Jacq.)	•••	••	•••	•••		R. s. U. S. to Ecuador.
<i>P. lutosus</i> Ridgw.	•	•	•			R. Guadelupe I.

TABLE III. VARIATION IN THE PICIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Campephilus principalis</i> (Linn.)	•	••		•	•	R. formerly s. Atlantic & Gulf St.
<i>C. p. bairdi</i> (Cass.)	•	•		•	•	R. Cuba.
<i>C. imperialis</i> (Gould)	••••	••		•	•••	R. w. Mex.
<i>C. guatemalensis</i> (Hartl.)	•••	•		•	•	R. s. Mex. to Costa Rica.
<i>Dryobates villosus</i> (Linn.)	••	••		•	•••	M. e. U. S. except s. St.
<i>D. v. leucomelas</i> (Bodd.)	•••	•		••	•	M. n. N. A. w. to Alaska.
<i>D. v. audubonii</i> (Swains.)	••	•		•	•	R. s. Atlantic & Gulf St.
<i>D. v. maynardi</i> Ridgw.	••••	••		••	•	R. Bahamas. [s. to Mex.
<i>D. v. harrisi</i> (Aud.)	••••	••		••	•••	M.? w. U. S. e. to Rocky Mts.,
<i>D. v. jardini</i> (Malh.)	•••	••••		••	••••	R. e. Mex. s. to Veracruz.
<i>D. pubescens</i> (Linn.)	••••	••		••	••	R. n. & e. N. A. [N. M.
<i>D. p. gairdnerii</i> (Aud.)	••	•••		••	•••	R. w. U. S., n. to B. C., s. to
<i>D. borealis</i> (Vieill.)	••	••		••	•	R. s. e. U. S. w. to Tex.
<i>D. scalaris</i> (Wagl.)	••	•		•	•	R. s. e. Mex.
<i>D. s. parvus</i> (Cabot)	••••	•		•	•	R. Yucatan.
<i>D. s. bairdi</i> (Scl.)	••	•		••	•	R. table-lands of Mex. to U. S.
<i>D. s. lucasanus</i> (Xantus)	•	•		•	•	R. s. L. Cal.
<i>D. s. sinaloensis</i> (Ridgw.)	••	•		•	•	R. w. Mex.
<i>D. s. graysoni</i> Baird	•••	•		•	•	R. Tres Marias Is.
<i>D. nuttallii</i> (Gamb.)	•	•		•	•••	R. Cal.
<i>D. arizonæ</i> (Hargitt)	••	•		••	•••	R. s. Ore. & n. w. Mex.
<i>Xenopicus albolarvatus</i> (Cass.)	••	•		•	•	R. mts. from Wash. Terr. to
<i>Picoides arcticus</i> (Swains.)	••	•		•	•	s. Cal. [U. S.
<i>P. americanus</i> Brehm	••	•		••••	•	R. n. N. A. s. to border of
<i>P. a. alascensis</i> (Nelson)	••	•		••••	•	M. n. N. A. e. of Rocky Mts.
<i>P. a. dorsalis</i> Baird	••••	•		••	•	M.? Alaska to Gt. Slave Lake.
<i>Sphyrapicus varius</i> (Linn.)	•	•		••	••	R.? Rocky Mts. from Kodiak
<i>S. v. nuchalis</i> Baird	•	•		•	•	to N. M.
<i>S. ruber</i> (Gmel.)	•	•		•	••	E. M. n. e. N. A. n. of U. S.
<i>S. thyroideus</i> (Cass.)	••••	•		•	•	M.? Rocky Mts. of U. S.
<i>Ceophloeus pileatus</i> (Linn.)	••••	••		••••	••	R. coast from Alaska to Cal.
<i>Melanerpes erythrocephalus</i> (Linn.)	•	•		•	•	R. w. U. S. to Rocky Mts.
<i>M. formicivorus</i> (Swains.)	••	••		•	•	R. whole of N. A.
<i>M. f. bairdi</i> Ridgw.	••••	••		••	••••	M. e. U. S.
<i>M. f. angustifrons</i> Baird	•	•		•	•	R. s. e. Mex. to Costa Rica.
<i>M. torquatus</i> (Wils.)	•	•		•	•	R. Mex. & contiguous U. S.
<i>M. elegans</i> (Swains.)	•••	•		•	•••	R. s. L. Cal.
<i>M. carolinus</i> (Linn.)	••••	••		••	••	R. w. U. S. e. to Rocky Mts.
<i>M. rubriventris</i> (Swains.)	•	•		•	•	R. s. & w. Mex.
<i>M. pygmaeus</i> Ridgw.	•	•		•	•	M. e. U. S. w. to Rocky Mts.
<i>M. aurifrons</i> (Wagl.)	•••	•		••	•	R. Yucatan.
<i>M. uropygialis</i> (Baird)	••••	•		••	•	R. Cozumel I.
<i>Colaptes auratus</i> (Linn.)	••	••••		•	••••	R. n. e. Mex. & s. Tex.
<i>C. chrysocaulosus</i> Gundl.	•••	••		•	••	R. s. Ariz. & Cal., L. Cal., w.
<i>C. chrysoides</i> (Malh.)	•••	••		•	••	Mex.
<i>C. cafer</i> (Gmel.)	••	••		•	•••	M. e. N. A. w. to Gt. Plains.
<i>C. c. saturator</i> Ridgw.	•••	••		••	••	R. Cuba. [Sonora.
<i>C. rufipileus</i> Ridgw.	•••	•		••	••	R. s. e. Cal., L. Cal., s. Ariz.,
						R.? w. U. S. & n. Mex. e. to
						Rocky Mts.
						R.? coast from Cal. to Sitka.
						R. Guadalupe I.

TABLE IV. VARIATION IN THE TYRANNIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Milvulus tyrannus</i> (Linn.) ♂	***			****	**	R. Mex. to S. A. & Lesser Antilles.
<i>Tyrannus tyrannus</i> (Linn.)	*	*		**	**	E. M. N. A. e. of Rocky Mts.
<i>T. magnirostris</i> D'Orb.	*	*		*	*	R. Cuba & Bahamas.
<i>T. dominicensis</i> (Gmel.)	*	*		**	***	R. W. Indies, coast of G. of Mex.
<i>T. crassirostris</i> Swains.	*	*			**	R. Mex.
<i>T. melancholicus couchi</i> (Baird)	***	**		**	***	R. Guatemala & Mex. to s. Tex.
<i>T. verticalis</i> Say	**	**		***	*	M. w. N. A. e. to Gt. Plains.
<i>T. vociferans</i> Swains.	*	*		*	**	R. Guatemala, Mex., L. Cal.
<i>Pitangus derbianus</i> (Kaup)	*	*		**	*	R. n. S. A. to Rio Grande.
<i>P. bahamensis</i> Bryant	*	*	*	*	*	R. Bahamas.
<i>Myiozetetes texensis</i> (Giraud)	*	*		*	*	R. Colombia to n. Mex.
<i>Myiodynastes luteiventris</i> Scl.	**	*		*	*	R. Mex. to Panama.
<i>M. audax</i> (Gmel.)	*	*		*	*	R. Cayenne, Trinidad, Tobago.
<i>M. a. nobilis</i> (Scl.)	***	*		**	*	R. Costa Rica s. to Ecuador.
<i>M. a. insolens</i> Ridgw.	*	*		****	*	R. s. e. Mex.
<i>Myiarchus mexicanus</i> (Kaup)	****	**	**	*	*	R. Guatemala n. to e. Mex.
<i>M. m. magister</i> Ridgw.	****	**	*	*	**	M. w. Mex. to s. Ariz.
<i>M. crinitus</i> (Linn.)	***	**	*	*	****	M. e. U. S. to Canada.
<i>M. cinerascens</i> Lawr.	***	**	*	*	***	M. w. U. S. e. to Rocky Mts.
<i>M. nuttingi</i> Ridgw.	****	*	*	*	**	R. s. Mex. to w. Costa Rica.
<i>M. brachyurus</i> Ridgw.	*	*	*	*	*	R. Nicaragua.
<i>M. yucatanensis</i> Lawr.	*	*	*	*	*	R. Yucatan.
<i>M. sagrae</i> Gundl.	*	*	*	*	*	R. Cuba.
<i>M. lucaysiensis</i> Bryant	*	*	*	*	*	R. Bahamas.
<i>M. lawrencei</i> (Giraud)	***	*	*	*	**	R. s. Tex. to Guatemala.
<i>M. l. olivascens</i> Ridgw.	**	**	*	*	*	M. w. Mex. to s. Ariz.
<i>M. flammulatus</i> Lawr.	*	*	*	*	*	R. s. w. Mex.
<i>Sayornis phoebe</i> (Lath.)	*	*		**	**	M. e. N. A. n. of Gulf St.
<i>S. nigricans</i> (Swains.)	*	*		**	*	M. coast from Ore. to Mex.
<i>S. saya</i> (Bonap.)	*	*		*	**	M. w. N. A. n. to Saskatchewan.
<i>Contopus borealis</i> (Swains.)	****	***	*	**	****	E. M. n. U. S. northward.
<i>C. pertinax</i> Cab.		***		*	*	R. Guatemala to s. Ariz.
<i>C. virens</i> (Linn.)	****	***	**	***	***	M. e. U. S. n. to Canada.
<i>C. richardsonii</i> (Swains.)	***	**	**	*	***	E. M. w. N. A. to int. of B. C.
<i>C. brachytarsus</i> Scl.	*	*	*	*	*	R. Yucatan & s. Mex.
<i>C. bahamensis</i> Bryant	*	*	*	*	**	R. Bahamas.
<i>C. caribæus</i> (D'Orb.)	*	*		*	*	R. Cuba.
<i>Empidonax albigularis</i> (Scl.)	**	*	*	*	*	R. s. e. Mex. & Guatemala.
<i>E. difficilis</i> Baird	♂	***	*	*	*	M. w. U. S. n. to Sitka.
<i>E. flaviventris</i> Baird	♂	***	*	*	*	E. M. n. U. S. northward.
<i>E. bairdii</i> Scl.	♂	*	*	*	*	R. s. & e. Mex.
<i>E. salvinii</i> (Ridgw.)	♂	*	***	*	****	R. highlands of Guatemala.
<i>E. acadicus</i> (Gmel.)	♂	**	**	**	***	E. M. e. U. S.
<i>E. pusillus</i> (Swains.)	♂	**	*	*	*	
	♂	**	**	*	**	E. M. w. N. A. to Sitka.

TABLE IV. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>E. p. traillii</i> (Aud.)	•	•••	•		•	E. M. n. U. S. northward.
<i>E. minimus</i> Baird	••	••	•••		••	E. M. n. U. S. northward.
<i>E. hammondi</i> (Xantus)	••	•	••	•	•	E. M. w. N. A. n. to L. Slave Lake.
<i>E. wrightii</i> Baird	••	••	•		••	M. w. U. S. to Rocky Mts.
<i>E. fulvipectus</i> Lawr.	•	•	•		•	R. s. Mex.
<i>E. fulvifrons rubicundus</i> (Cab. & Hein.)	•	•	••		••	R. s. Mex.
<i>E. f. pygmæus</i> (Coues)	•	••	•••	•	•	R. s. Ariz. to w. Mex.
<i>Pyrocephalus rubineus mexicanus</i> (Scl.)		•		••	•	R. Guatemala to s. U. S.
<i>Ornithion imberbe</i> (Scl.)	•	•	•		••	R. C. A. to s. Tex.
<i>O. i. ridgwayi</i> Brewst.	•	••	••	••	••	R. ? w. Mex. to s. Ariz.

TABLE V. VARIATION IN THE CORVIDÆ.

<i>Pica pica</i> (Linn.)	••	•	••		••••	R. n. & c. Europe.
<i>P. p. hudsonica</i> (Sab.)	••••	•••	••	••••	••••	R. w. N. A. from N. M. to Ariz.
<i>P. nuttalli</i> Aud.	••	•	•••	••	••	R. Cal.
<i>Psilorhinus morio</i> (Wagl.)		•		••	••	R. e. Mex.
<i>P. cyanogenys</i> Gray				••	•	R. e. Mex. & coast of Honduras.
<i>P. mexicanus</i> Rüpp.		••		•	•••	R. s. Mex. to Costa Rica.
<i>Cyanocitta cristata</i> (Linn.)	••	••	•••	••	••	R. e. N. A. n. to fur countries.
<i>C. c. florincola</i> Coues.	••	••	••	•••	••	R. Fla.
<i>C. stelleri</i> (Gmel.)	••••	••	•••	•	•••	R. n. w. coast from Cal. to Sitka.
<i>C. s. frontalis</i> Ridgw.	••••	••	•	••	••	R. Sierra Nevada.
<i>C. s. annectens</i> (Baird)	••	••	•	••	••	R. n. Rocky Mts.
<i>C. s. macrolopha</i> (Baird)	••	••	•	•••	•••	R. s. Rocky Mts. to n. Mex.
<i>C. s. diademata</i> (Bonap.)	••	•	•		•	R. highlands of c. Mex.
<i>C. s. coronata</i> (Swains.)	••	•	•		•••	R. s. Mex. to Guatemala.
<i>Aphelocoma floridana</i> (Bartr.)	••	•	•••	•••	••	R. Fla.
<i>A. woodhousei</i> (Baird.)	••	••	•	••	•••	R. middle province of U. S. s. to Mex.
<i>A. insularis</i> Hensh.	••	•	•	•	•	R. Santa Cruz I.
<i>A. californica</i> (Vig.)	•••	••	••••	•	••	R. coast from s. Cal. to Ore.
<i>A. c. hypoleuca</i> (Ridgw.)	••	•	••	•	••	R. s. L. Cal.
<i>A. sumichrasti</i> Ridgw.		•			••	R. s. Mex.
<i>A. couchi</i> (Baird)	•	•			•	R. L. Rio Grande vall.
<i>A. sieberii</i> (Wagl.)	•	••	•	••	•	R. s. Mex. & southward.
<i>A. s. arizonæ</i> Ridgw.	•	•	•	••	•	R. n. w. Mex. & adjacent Ariz. & N. M.
<i>Xanthoura luxuosa</i> (Less.)		•		•	••	R. e. Mex.
<i>Perisoreus canadensis</i> (Linn.)	••	•	••	••	••	R. N. E. to Minn., n. to arctic regions.

TABLE V. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>P. c. nigricapillus</i> Ridgw.	**	*	*	*	**	R. coast region of Labrador.
<i>P. c. fumifrons</i> Ridgw.	**	**	*	****	***	R. Alaska.
<i>P. c. capitalis</i> Baird	**	*	*	***	*	R. Rocky Mts. from Ore. into B. A.
<i>P. obscurus</i> (Ridgw.)	***	**	**	***	**	R. n. Cal. & n. Sierra Nevada to B. C.
<i>Corvus corax</i> Linn.	**	*	*	*	*	R. Eurasia.
<i>C. c. sinuatus</i> (Wagl.)	****	***	***	****	****	R. w. U. S. to Guatemala.
<i>C. c. principalis</i> Ridgw.	****	*	**	****	**	R. n. N. A. from Greenland to Alaska.
<i>C. c. behringianus</i> Dybowski	**	*	*	*	*	R. Commander Is.
<i>C. cryptoleucus</i> Couch.	***	*	**	**	**	R. s. w. U. S. & table-lands of Mex.
<i>C. americanus</i> Aud.	**	**	*	****	***	R. e. N. A. except arctic dists.
<i>C. a. floridanus</i> Baird	**	*	*	*	**	R. s. Fla.
<i>C. caurinus</i> Baird	***	*	**	*	***	R. Wash. Terr. to Kodiak.
<i>C. ossifragus</i> Wils.	**	**	***	***	***	R. coast from Long I. to La.
<i>C. mexicanus</i> Gmel.	**	*	**	****	**	R. w. Mex.
<i>Nucifraga columbiana</i> (Wils.)		**	*	*	*	R. w. N. A. from Ariz. to Alaska.
<i>Cyanocephalus cyanocephalus</i> (Wied)		*		***	*	R. w. N. A. between Rocky Mts. & Sierra Nevada.

TABLE VI. VARIATION IN THE ICTERIDÆ.

<i>Dolichonyx oryzivorus</i> (Linn.)	♂	*	*	*	*	E. M. e. N. A. in U. S. w. to Gt. Plains.
<i>Molothrus ater</i> (Bodd.)	♂	***	***	**	*	M. U. S. & s. Can.
<i>M. a. obscurus</i> (Gmel.)	♂	**	**	*	*	R. Mex., L. Cal., contiguous U. S.
<i>Callothrux æneus</i> (Wagl.)	♂	*	*	*	*	R. Rio Grande to Panama.
<i>Xanthocephalus xanthocephalus</i> (Bonap.)	♂	*	*	*	*	M. marshes of w. U. S.
<i>Agelaius phœniceus</i> (Linn.)	♂	****	***	****	****	M. nearly whole temp. N. A.
<i>A. p. sonoriensis</i> Ridgw.	♂	**	*	**	*	R. n. w. Mex., s. Cal., lower Col. val.
<i>A. p. bryanti</i> Ridgw.	♂	*	*	*	*	R. Bahamas & s. Fla.
<i>A. gubernator</i> (Wagl.)	♂	****	***	*	***	R. vall. of Ore. & Cal. into Mex.
<i>A. assimilis</i> Gundl.	♂	*	*	*	*	R. Cuba.

TABLE VI. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
A. tricolor (Nutt.)	♂	*	*	*	*	R. coast vall. from s. Cal. to w. Ore.
Sturnella magna (Linn.)	♀	*	*	*	****	R. e. N. A. n. to Canada, w. to Gt. Plains.
S. m. neglecta (Aud.)	♀	**	*	*		R. w. N. A. from Manitoba to w. Mex.
S. m. mexicana (Scl.)	♀	**	*	*		R. s. Ariz. & e. Mex. to Costa Rica.
Quiscalus quiscula (Linn.)	♀	*	*	*	****	M. Atlantic slope of U. S. from N. E. southward.
Q. q. aglæus (Baird)	♂	*	*	*	***	R. G. coast from Fla. to La.
Q. q. æneus (Ridgw.)	♂	**	*	*	**	M. c. N. A. n. to N. E.
Q. macrourus Swains.	♂	**	*	*	**	R. s. Tex. to Nicaragua.
Q. graysoni Scl.	♂	*	**	*	****	R. w. Mex.
Q. major (Vieill.)	♂	**	*	*	**	R. s. Atlantic & G. coast of U. S.
Q. tenuirostris Swains.	♀	*	*	*	***	R. c. Mex.

TABLE VII. VARIATION IN THE FRINGILLIDÆ.

Coccothraustes vespertina (Coop.)	*	*	*	****	***	I. M. w. N. A. n. to B. C.
Pinicola enucleator (Linn.)	***	*	**	*	*	I. M. n. Eurasia.
Pyrrhula cassini (Baird)		*			*	M. ? n. Alaska & portions of Siberia.
Carpodacus purpureus (Gmel.)	♂	*	*	*	*	M. e. U. S. northward.
C. p. californicus (Baird)	♂	*	*	*	*	M. coast from s. Cal. to B. C.
C. cassini Baird		*	*	*	***	M. w. U. S. from B. C. to Mex.
C. mexicanus (Müll.)	♂	*	*	*	*	R. e. & s. Mex.
C. m. frontalis (Say)	♂	***	*	*	***	M. w. U. S. from 40° lat. to Mex.
C. amplus Ridgw.	**	*	**	*	**	R. Guadelupe I.
Loxia curvirostra minor (Brehm.)	****	**	***	**	****	M. n. N. A. e. of Gt. Plains.
L. c. stricklandi (Ridgw.)	**	*	**	*	*	R. s. w. U. S. & Mex.
L. leucoptera Gmel.				*	*	M. n. U. S. northward.
Leucosticte griseonucha (Brandt)	****	***	***	**	****	M. ? Aleutian, Prybilof & Commander Is.

TABLE VII. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>L. tephrocotis littoralis</i> (Baird)	***	**	**	**	***	M. coast mt. ranges of n. w. N. A.
<i>L. atrata</i> Ridgw.	**	**	*	*	**	M. (summer range unknown).
<i>L. australis</i> (Allen)	***	**	**	**	***	M. high mts. of Col.
<i>L. arctoa</i> (Brandt)				*		M. ? n. e. Asia.
<i>Acanthis hornemannii</i> (Holb.)	♂♂	**	**		*	M. n. Greenland & e. Arctic A.
<i>A. h. exilipes</i> (Coues)	♂♂	*	**		*	M. circumpolar continental regions.
<i>A. linaria</i> (Linn.)	♂♂	*	**		**	M. n. portions of N. Hemisphere.
<i>A. l. holbælli</i> (Brehm)	♂♂	*	*		*	M. n. coasts of Eurasia, portions of Alaska.
<i>A. l. rostrata</i> (Coues)	♂♂	*	*		**	M. s. Greenland.
<i>Spinus psaltria</i> (Say)	*			**	**	M. w. U. S. from n. Cal. to Col.
<i>S. lawrencei</i> (Cass.)		**		*	**	M. Cal.
<i>S. notatus</i> (Du Bus.)		**		*	***	R. highlands of s. Mex. & Guatemala.
<i>S. atriceps</i> (Salv.)				*	*	R. Guatemala.
<i>S. pinus</i> (Wils.)				***	*	E. M. n. U. S. northward.
<i>Carduelis carduelis</i> (Linn.)	***	*		***	*	R. Eurasia.
<i>Plectrophenax nivalis</i> (Linn.)	♂	*	*	*	**	M. circumpolar regions.
<i>P. n. townsendi</i> Ridgw.	♂	**	**		**	R. Alaska, Prybilof, & Commander Is.
<i>P. hyperboreus</i> Ridgw.	♂	*	*	*	*	M. Hall I.
<i>Calcarius lapponicus</i> (Linn.)	♂♂	*	*	*	**	E. M. circumpolar regions.
<i>C. pictus</i> (Swains.)	♂♂	*	*	*	*	E. M. int. of Arctic A.
<i>C. ornatus</i> (Townsend)	♂	*				M. Gt. Plains n. to Saskatchewan.
<i>Poocætes gramineus</i> (Gmel.)	***	***	*	***	**	M. e. N. A. from Va. to Ont.
<i>P. g. confinis</i> (Baird)	***	***	*	**	***	M. w. N. A. from B. C. southward.
<i>Ammodramus princeps</i> (Mayn.)	***	**	**	**	**	M. Sable I.
<i>A. sandvichensis</i> (Gmel.)	***	**	**	*	**	M. n. w. coast from Unalaska south.
<i>A. s. savanna</i> (Wils.)	**	**	***	**	***	M. n. U. S. to Labr.
<i>A. s. alaudinus</i> (Bonap.)	*	*	**	***	***	E. M. w. N. A. n. to Alaska.
<i>A. s. bryanti</i> Ridgw.	*	**	***		**	R. salt marshes of San Francisco Bay.
<i>A. beldingi</i> Ridgw.	***	**	*	*	***	R. salt marshes of s. Cal. & L. Cal.

TABLE VII. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>A. rostratus</i> Cass.	*	***	***		***	M. coasts of s. Cal., L. Cal., & Sonora.
<i>A. r. guttatus</i> (Lawr.)				*		R. Cape St. Lucas.
<i>A. bairdii</i> (Aud.)		***		**		M. Gt. Plains from Da. to Saskatchewan.
<i>A. savannarum passerinus</i> (Wils.)	***	**	*	*	**	M. e. U. S. & s. Canada.
<i>A. s. perpallidus</i> Ridgw.	***	*	*	**	**	M. w. U. S. e. to Gt. Plains.
<i>A. henslowii</i> (Aud.)		*		*	*	M. e. U. S. to Gt. Plains, n. to Ont.
<i>A. lecontei</i> (Aud.)		**		****	****	E. M. Gt. Plains from Da. to Manitoba.
<i>A. caudacutus</i> (Gmel.)	+	**	*	*	***	M. coast from Me. to N. Ca.
<i>A. c. nelsoni</i> Allen	+	*	*	*	**	M. marshes of Miss. vall.
<i>A. maritimus</i> (Wils.)	+	**	*	*	*	M. coast from Mass. to Tex.
<i>A. nigrescens</i> Ridgw.	****	*	*	*	***	R. s. e. Fla.
<i>Chondestes grammacus</i> (Say)		***		***	**	M. Miss. vall. n. to Mich.
<i>C. g. strigatus</i> (Swains.)		**		**	****	M. w. U. S. e. to Gt. Plains.
<i>Zonotrichia querula</i> (Nutt.)		**		**	*	M. e. Gt. Plains from Mon. to Manitoba.
<i>Z. leucophrys</i> (Forst.)	****	**	*	***	**	E. M. high mts. of w. U. S. to Labr.
<i>Z. l. intermedia</i> Ridgw.	**	**	*	***	*	E. M. Alaska & Mackenzie r. basin.
<i>Z. l. gambeli</i> (Nutt.)	****	***	****	***	***	M. coast mts. of Cal. to B. C.
<i>Z. coronata</i> (Pall.)		**		**	*	M. n. Cal. to Norton Sound.
<i>Z. albicollis</i> (Gmel.)		**		****	*	M. n. U. S. northward.
<i>Spizella monticola</i> (Gmel.)		**		*	*	M. Labr. & Hudson B. region.
<i>S. m. ochracea</i> (Brewst.)		**		**	***	E. M. Alaska.
<i>S. socialis</i> (Wils.)		**		***	***	M. e. N. A. to Gt. Slave Lake.
<i>S. s. arizonæ</i> Coues		*		**	**	E. M. w. N. A. n. to 60° lat.
<i>S. pusilla</i> (Wils.)		**		***	**	M. e. U. S. & s. Canada, w. to Gt. Plains.
<i>S. p. arenacea</i> Chadb.		*		*	**	M. Gt. Plains from Tex. to Wyoming.
<i>S. pallida</i> (Swains.)		**		***	**	E. M. Gt. Plains n. to Saskatchewan.
<i>S. breweri</i> Cass.		*		**	**	M. w. U. S. e. to Rocky Mts.
<i>S. atrigularis</i> (Cab.)		*		*	*	R. Mex., L. Cal.
<i>Junco aikeni</i> (Ridgw.)	+	*	***	*	*	I. M. Rocky Mts. in Col. & Wy.
<i>J. hyemalis</i> (Linn.)	+	**	*	*	*	M. Me. to Alaska.
<i>J. h. carolinensis</i> Brewst.	+	*	*	*	*	R. s. Alleghany Mts.
<i>J. h. oregonus</i> (Townsend.)	+	**	*	*	*	M. coast from Cal. to Sitka.
<i>J. caniceps</i> (Woodh.)	+	*	*	*	**	M. Rocky Mt. distr.
<i>J. cinereus</i> (Swains.)	+	*	***	*	**	R. highlands of Mex.

TABLE VII. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>J. c. dorsalis</i> (Henry)	••	••	••	••	••	R. s. Rocky Mts.
<i>J. c. palliatus</i> Ridgw.	••	••	••	••	••	R. s. Ariz. & adjacent Mex.
<i>J. alticola</i> Salv.	••	••	••	••	••	R. highlands of Mex.
<i>J. annectens</i> (Baird)	••	••	••	••	••	M. Ft. Bridger northward.
<i>J. insularis</i> Ridgw.	••	••	••	••	••	R. Guadalupe I.
<i>J. bairdi</i> Belding	••	••	••	••	••	R. mts. of s. L. Cal.
<i>Amphispiza bilineata</i> (Cass.)	••	••	••	••	••	M. s. w. U. S. & Mex.
<i>A. mystacalis</i> (Hartl.)	••	••	••	••	••	R. s. Mex.
<i>A. humeralis</i> (Cab.)	••	••	••	••	••	R. s. Mex.
<i>A. belli</i> (Cass.)	••	••	••	••	••	R. Cal. to Cape St. Lucas.
<i>A. b. nevadensis</i> (Ridgw.)	••	••	••	••	••	M. w. U. S. from Mex. to Mon.
<i>Pencæa æstivalis</i> (Licht.)	••	••	••	••	••	R. Fla. & lower Ga.
<i>P. a. bachmani</i> (Aud.)	••	••	••	••	••	R. s. Atlantic & G. St.
<i>P. mexicana</i> (Lawr.)	••	••	••	••	••	R. Mex.
<i>P. botteri</i> Scl.	••	••	••	••	••	R. s. e. Mex.
<i>P. cassini</i> (Woodh.)	••	••	••	••	••	M. s. w. border of U. S.
<i>P. ruficeps</i> (Cass.)	••	••	••	••	••	R. Cal.
<i>P. r. boucardi</i> (Scl.)	••	••	••	••	••	R. Mex., s. Ariz., N. M., L. Cal.
<i>P. carpalis</i> Coues	••	••	••	••	••	R. s. Ariz.
<i>P. notosticta</i> Scl. & Salv.	••	••	••	••	••	R. s. Mex.
<i>Melospiza fasciata</i> (Gmel.)	••	••	••	••	••	R. e. U. S. & Brit. Prov. n. of 40° lat.
<i>M. f. montana</i> (Hensh.)	••	••	••	••	••	R. Rocky Mts. w. to Ore. & Nev.
<i>M. f. heermanni</i> (Baird)	••	••	••	••	••	R. int. of Cal.
<i>M. f. samuelis</i> (Baird)	••	••	••	••	••	R. coast of Cal.
<i>M. f. mexicana</i> Ridgw.	••	••	••	••	••	R. s. Mex.
<i>M. f. fallax</i> (Baird)	••	••	••	••	••	R. Ariz.
<i>M. f. guttata</i> (Nutt.)	••	••	••	••	••	R. coast from Ore. to Vancouver.
<i>M. f. rufina</i> (Bonap.)	••	••	••	••	••	R. coast of s. Alaska.
<i>M. cinerea</i> (Gmel.)	••	••	••	••	••	R. Aleutian Is.
<i>M. georgiana</i> (Lath.)	••	••	••	••	••	M. e. U. S. to Labr.
<i>M. lincolni</i> (Aud.)	••	••	••	••	••	E. M. n. U. S. northward.
<i>Passerella iliaca</i> (Merr.)	••	••	••	••	••	E. M. G. of St. Lawrence to Labr. & Alaska.
<i>P. i. unalaschcensis</i> (Gmel.)	••	••	••	••	••	E. M. coast of Alaska.
<i>P. i. negarhyncha</i> (Baird)	••	••	••	••	••	R. mts. of Cal.
<i>P. i. schistacea</i> (Baird)	••	••	••	••	••	M. Rocky Mts.
<i>Embernagra rufivirgata</i> Lawr.	••	••	••	••	••	R. Rio Grande vall. southward.
<i>E. r. crassirostris</i> Baird	••	••	••	••	••	R. s. Mex.
<i>E. r. verticalis</i> Ridgw.	••	••	••	••	••	R. Yucatan.
<i>Pipilo erythrophthalmus</i> (Linn.)	••	••	••	••	••	M. s. St. to B. A.
<i>P. e. alleni</i> Coues	••	••	••	••	••	R. Fla.
<i>P. maculatus</i> Swains.	••	••	••	••	••	R. c. Mex. to Guatemala.

TABLE VII. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>P. m. arcticus</i> (Swains.)	**	*	*	*	*	M. Gt. Plains to Saskatchewan.
<i>P. m. megalonyx</i> (Baird)	***	*	*	*	**	M. Rocky Mts. from L. Cal. to Wash.
<i>P. m. oregonus</i> (Bell)	**	*	*	*	**	M. coast from Cal. to B. C.
<i>P. consobrinus</i> Ridgw.		**	**	***		R. Guadelupe I.
<i>P. carmani</i> Lawr.	*	*	*	*	*	R. Socorro I.
<i>P. macronyx</i> Swains.	*	*	*	*	*	R. vall. of Mex.
<i>P. chlorosoma</i> Baird		*	*	*	*	R. s. Mex.
<i>P. chlorurus</i> (Townes.)		**		**	**	M. Rocky Mts. n. to Ore.
<i>P. rutilus</i> Licht.				*	*	R. s. Mex.
<i>P. fuscus</i> Swains.	*	**	**		***	R. Mex.
<i>P. f. mesoleucus</i> (Baird)	**	**	*	*	*	R. N. M. & s. Ariz.
<i>P. f. albigula</i> (Baird)	**	*	**	*	**	R. L. Cal.
<i>P. f. crissalis</i> (Vig.)	**	*	*	*	*	R. Cal.
<i>P. aberti</i> Baird		**		*	*	R. N. M. & Ariz. to Col.
<i>Cardinalis cardinalis</i> (Linn.) ♂	**	**	**	***		R. e. U. S. n. to 40° lat.
<i>C. c. superbus</i> Ridgw.	♂	*	*	*	*	R. w. Mex. to s. Ariz.
<i>C. c. igneus</i> (Baird)	♂	*	**	*	*	R. L. Cal.
<i>C. c. coccineus</i> Ridgw.	♂	*	*	*	*	R. e. & c. Mex.
<i>C. c. yucatanicus</i> Ridgw.	♂	*	*	*	*	R. Yucatan.
<i>C. c. saturatus</i> Ridgw.	♂	*	*	*	*	R. Cozumel I.
<i>C. carneus</i> (Less.)	*	*	*	*	*	R. s. w. Mex.
<i>C. phœniceus</i> Gould	♂				***	R. n. coast of S. A.
<i>Pyrrhuloxia sinuata</i> Bonap.		*		***	**	R. Mex. & contiguous U. S.
<i>Habia ludoviciana</i> (Linn.)		*		****	*	E. M. n. U. S. & Canada.
<i>H. melanocephala</i> (Swains.)		*		***	**	M. w. U. S. e. to Gt. Plains.
<i>Guiraca cærulea</i> (Linn.) ♂	*	*	*	*	*	M. s. e. U. S.
<i>G. c. eurhyncha</i> Coues	♂	*	*	*	*	M. w. U. S. n. to Col. & Cal.
<i>G. cyanoides concreta</i> (Du Bus)	**	**	*	***		R. C. A. to e. Mex.
<i>Passerina parellina</i> (Bonap.)	**	*	*	*	*	R. s. & e. Mex.
<i>P. amœna</i> (Say)		*		****	****	M. w. U. S. e. to Gt. Plains.
<i>P. cyanea</i> (Linn.)		*		****	**	M. e. U. S. & s. Canada to Gt. Plains.
<i>P. versicolor</i> (Bonap.)	♂	*	*	*	*	R. e. Mex. to Tex.
<i>P. v. pulchra</i> Ridgw.	♂	*	*	**	**	R. L. Cal. & w. Mex.
<i>P. ciris</i> (Linn.)		*		****	*	M. s. Atlantic & Gulf St.
<i>P. leclancheri</i> Lafr.		**	**	****	*	R. s. w. Mex.
<i>P. rosiæ</i> (Lawr.)	*	*	*	*	*	R. s. Mex.
<i>Sporophila moreletti</i> (Bonap.)	**			****	**	R. Rio Grande to Costa Rica.
<i>S. torqueola</i> Bonap.	*	*	*	*	*	R. w. Mex.
<i>S. corvina</i> Scl.	*	*	*	*	**	R. e. Mex. to Costa Rica.
<i>Euethia bicolor</i> (Linn.)	*	*	*	*	*	R. Bahamas.
<i>Spiza americana</i> (Gmel.)	***			***	****	E. M. e. U. S. to Rocky Mts.
<i>Calamospiza melanocorys</i> Stejn.	**			****	***	M. Gt. Plains from Kan. to beyond U. S.

TABLE VIII. VARIATION IN THE VIREONIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Vireo altiloquus barbatulus</i> (Cab.)	**	*		*	**	R. Cuba, Bahamas, s. Fla.
<i>V. olivaceus</i> (Linn.)	**	*		***	*	E. M. e. N. A. n. to Hudson B. w. to Rocky Mts.
<i>V. flavoviridis</i> (Cass.)	*	**		*	****	R. Rio Grande to Upper Amazon.
<i>V. cinereus</i> Ridgw.	*	*				R. Cozumel I.
<i>V. philadelphicus</i> (Cass.)	*	**			***	E. M. e. N. A., chiefly n. of U. S.
<i>V. gilvus</i> (Vieill.)	*	**	*	**	**	E. M. e. N. A. n. to Hudson B. w. to Gt. Plains.
<i>V. g. swainsoni</i> (Baird)	*	**	*	**	***	E. M. w. U. S. e. to Rocky Mts.
<i>V. flavifrons</i> Vieill.		*		***	***	E. M. e. U. S. n. of middle Sts. w. to Gt. Plains.
<i>V. solitarius</i> (Wils.)	*	*	*	****	*	E. M. e. N. A., chiefly n. of U. S.
<i>V. s. cassinii</i> (Xantus)	**	*	**	**	*	M. w. U. S., chiefly on Pacific coast.
<i>V. s. alticola</i> Brewst.	****	*	*	*	*	M. higher s. Alleghany Mts.
<i>V. s. plumbeus</i> (Coues)	***	*	*	*	**	M. Rocky Mt. dist. of U. S.
<i>V. atricapillus</i> Woodh.		*	*	*	**	M. s. Gt. Plains n. to Kan.
<i>V. noveboracensis</i> (Gmel.)	*	*	*	**	*	E. M. e. U. S. w. to Rocky Mts.
<i>V. n. maynardi</i> Brewst.	*	*	*	*	*	R. Key West.
<i>V. bellii</i> (Aud.)	*	**	*	****	*	M. Gt. Plains n. to Da.
<i>V. b. pusillus</i> (Coues)	***	*	**	*	*	R. s. Cal., L. Cal., Ariz.
<i>V. ochraceus</i> Salv.	**	*	*	*	*	R. Yucatan to Guatemala.
<i>V. huttoni</i> Cass.	**	*	*	**	*	R. Cal.
<i>V. h. stephensi</i> Brewst.	****	*	*	*	*	R. Mex., Tex., L. Cal., Ariz.
<i>V. pallens</i> Salv.		*			*	R. w. coast of Costa Rica & Nicaragua.
<i>V. vicinior</i> Coues		*	**	*	*	R. s. Cal., Ariz., N. M., n. w. Mex.
<i>V. gundlachi</i> Lemb.		*	**		*	R. Cuba.
<i>V. hypochryseus</i> Scl.		*			*	R. s. w. Mex.
<i>Hylophilus decurtatus</i> (Bonap.)	**				*	R. e. Mex. & Guatemala.
<i>H. ochraceiceps</i> Scl.		*			*	R. s. Mex. s. to Costa Rica.

TABLE IX. VARIATION IN THE MNIOTILTIDÆ.

<i>Mniotilta varia</i> (Linn.)		**		****	***	E. M. e. N. A. from Potomac r. to Hudson Bay.
<i>Protonotaria citrea</i> (Bodd.)		*		**	*	M. Gulf St. & lower Miss. vall.
<i>Helinaia swainsonii</i> Aud.	**	***	***	****	***	M. Gulf St. & lower Miss. vall.
<i>Helmitherus vermivorus</i> (Gmel.) ♂	*	*		***	***	M. e. U. S. n. to about 40°.
<i>Helminthophila celata</i> (Say)		*		***	*	E. M. n. N. A. from Rocky Mts. to Alaska.

TABLE IX. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>H. c. lutescens</i> Ridgw.		*		*	*	M. coast from s. Cal. to Kodiak.
<i>H. ruficapilla</i> (Wils.)	♂	*		***	*	E. M. e. N. A. from n. U. S. to Hudson Bay.
<i>H. r. gutturalis</i> Ridgw.	♂	*			*	M. w. U. S. from Rocky Mts. to coast.
<i>H. virginæ</i> (Baird)		*		*	*	M. mts. of w. U. S.
<i>H. luciae</i> (Coop.)		*		***	**	R. Ariz., s. e. Cal., Sonora.
<i>Compsothlypis americana</i> (Linn.)		*		****	***	E. M. e. U. S. to Canada.
<i>C. nigrilora</i> (Coues)		**		**	**	R. lower Rio Grande vall.
<i>C. insularis</i> (Lawr.)		*		*	*	R. Tres Marias Is.
<i>C. graysoni</i> Ridgw.		*		*	*	R. Socorro I.
<i>Dendroica tigrina</i> (Gmel.)				****		E. M. n. N. A. to Hudson Bay.
<i>D. olivacea</i> (Giraud)				***		R. Tex. to Guatemala.
<i>D. æstiva</i> (Gmel.)		**		***	***	E. M. e. & n. N. A.
<i>D. petechia</i> (Linn.)	♂	*		*	**	R. W. Indies, Cozumel I.
<i>D. cærulescens</i> (Gmel.)		*		***	*	E. M. e. N. A. from n. N. E. northward.
<i>D. coronata</i> (Linn.)		*		****	*	M. n. U. S. northward.
<i>D. audubonii</i> (Townsend)		**		***	*	E. M. w. N. A. n. to B. C.
<i>D. maculosa</i> (Gmel.)		*		**	**	E. M. n. N. E. to Hudson Bay.
<i>D. cærulea</i> (Wils.)		**		****	**	E. M. Miss. vall. to Alleghenies.
<i>D. pennsylvanica</i> (Linn.)		**		**	*	E. M. e. U. S. & Canada n. of 40° lat.
<i>D. castanea</i> (Wils.)		*		****	*	E. M. n. N. E. to Hudson Bay.
<i>D. striata</i> (Forst.)		*		***	*	E. M. n. N. E. & Labr. to Alaska.
<i>D. dominica</i> (Linn.)	****	**		*	***	M. s. Atlantic St.
<i>D. d. albilora</i> Baird	****	*		****	**	M. Miss. vall. n. to Gt. Lakes.
<i>D. blackburniæ</i> (Gmel.)		**		****	**	E. M. e. N. A. from n. U. S. northward.
<i>D. graciae</i> Coues		**		*	*	M. s. Ariz. & N. M., Mex.
<i>D. decora</i> (Ridgw.)				**	*	R. s. Mex. & Guatemala.
<i>D. nigrescens</i> (Townsend)		**		**	*	M. w. U. S. n. to Ore. & Col.
<i>D. chrysoparia</i> Scl. & Salv.				***	*	R. c. Tex. & highlands of Guatemala.
<i>D. virens</i> (Gmel.)		*		****	*	E. M. n. e. U. S. northward.
<i>D. townsendi</i> (Nutt.)		**		*	**	E. M. w. N. A. n. to Sitka.
<i>D. occidentalis</i> (Townsend)		**		**	*	M. w. U. S. near coast.
<i>D. vigorsii</i> (Aud.)		**		**	***	M. e. U. S. n. to Ont.
<i>D. kirtlandi</i> Baird		**		**	**	M.
<i>D. discolor</i> (Vieill.)		*		***	*	M. e. U. S. n. to Mich. & s. N. E.
<i>D. palmarum</i> (Gmel.)		**	**	****	***	E. M. int. of N. A., n. to Gt. Slave Lake.
<i>D. p. hypochrysea</i> Ridgw.		**	*	***	**	M. coast from Nova Scotia to Hudson Bay.
<i>Seiurus aurocapillus</i> (Linn.)		*		****	****	E. M. e. N. A. to Alaska.
<i>S. noveboracensis</i> (Gmel.)		**	**	****	**	E. M. n. e. U. S. northward.
<i>S. n. notabilis</i> (Grinn.)	****	**	*	***	***	E. M. w. N. A. to Miss. r. & Alaska.

TABLE IX. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>S. motacilla</i> (Vieill.)	**	*	*	**	*	M. e. U. S. n. to Gt. Lakes.
<i>Geothlypis formosa</i> (Wils.)		*	**	***	***	E. M. e. U. S. to s. N. E.
<i>G. agilis</i> (Wils.)		**	****	***	***	E. M. Manitoba.
<i>G. philadelphia</i> (Wils.)	♂	**	*		**	E. M. N. E. northward.
<i>G. macgillivrayi</i> (Aud.)	♂	***			**	
<i>G. trichas</i> (Linn.)	♂	*	*		****	E. M. Mts. of w. N. A. n. to B. C.
<i>G. t. occidentalis</i> Brewst.	**	**	**	****	**	M. e. U. S. n. to Canada.
<i>G. melanops</i> Baird		**	*			M. w. U. S.
<i>G. beldingi</i> Ridgw.	*	**	*		**	R. e. & s. Mex.
<i>G. rostrata</i> Bryant	*	**	*		*	R. s. L. Cal.
<i>G. tanneri</i> Ridgw.	*	**	*		**	R. New Providence I.
<i>G. coryi</i> Ridgw.	*	*	*		*	R. Obaco I.
<i>G. speciosa</i> Scl.		*	*		*	R. Eleuthera I.
<i>G. poliocephala</i> Baird		*	*		*	R. s. e. Mex.
<i>G. palpebralis</i> Ridgw.	*	*	*		*	R. w. Mex.
<i>G. caninucha</i> Ridgw.	*	*	*		*	R. e. Mex. & Yucatan.
<i>Icteria virens</i> (Linn.)	♂	**			***	R. Guatemala to Costa Rica.
<i>I. v. longicauda</i> (Lawr.)	♂	*	*		*	M. e. U. S. n. to Ont.
<i>Sylvania mitrata</i> (Gmel.)	♂	**	**	*	*	M. w. U. S.
<i>S. pusilla</i> (Wils.)	♂	**			*	M. e. U. S. n. to N. E.
<i>S. p. pileolata</i> (Pall.)	♂	*	*		**	E. M. n. e. U. S. northward.
<i>S. canadensis</i> (Linn.)		*	***	*	*	E. M. w. N. A. to Kodiak.
<i>Setophaga ruticilla</i> (Linn.)		*	****	*	*	E. M. e. N. A. n. to L. Winni-peg.
<i>Cardellina rubrifrons</i> (Giraud)	**		**	**	**	E. M. e. N. A.
<i>Ergaticus ruber</i> (Swains.)		*	**	*	*	R. highlands of Guatemala to s. Ariz.
<i>Basileuterus culicivorus</i> (Licht.)		*	**	*	*	R. highlands of e. Mex.
<i>B. belli</i> (Giraud)		**	*	*	*	R. Veragua to e. Mex.
<i>B. delatrii</i> Bonap.	*	**	**	***	*	R. Guatemala & e. Mex.
<i>B. rubrifrons</i> (Swains.)	*	*	*	*	*	R. Guatemala to Panama.
					*	R. s. Mex.

TABLE X. VARIATION IN THE TROGLODYTIDÆ.

<i>Oroscoptes montanus</i> (Towns.)	*	*	*	**	*	M. Artemisia Plains of w. U. S.
<i>Mimus polyglottos</i> (Linn.)	***	***	***	****	***	R. 38° lat. to Mex. & Bahamas.
<i>M. lawrencei</i> Ridgw.	*	*	*	*	*	R. s. Mex.
<i>M. gracilis</i> Cab.	*	***	**		***	R. Atlantic coast from Yucatan to Honduras.
<i>M. gundlachi</i> Cab.	***	*	**		*	R. Bahamas, Cuba, Jamaica.
<i>Galeoscoptes carolinensis</i> (Linn.)	****	*	*	*	*	E. M. e. N. A. n. to 54° lat.
<i>Mimodes graysoni</i> (Baird)	****	*	*	*	*	R. Socorro I.
<i>Harporhynchus rufus</i> (Linn.)	****	**	*	**	***	M. e. U. S. to Rocky Mts., n. to Ont.

TABLE X. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>H. longirostris</i> (Lafr.)	****	**	*	**	**	R. e. Mex. & s. Tex.
<i>H. guttatus</i> Ridgw.	*	*	*	*	*	R. Cozumel I.
<i>H. cinereus</i> Xantus	*	*	**	*	*	R. L. Cal.
<i>H. bendirei</i> Coues	*	*	*	**	***	R. s. Ariz.
<i>H. curvirostris</i> (Swains.)	***	*	*	*	*	R. Mex., s. Tex. & s. N. M.
<i>H. c. palmeri</i> Ridgw.	***	*	**	*	*	R. s. Ariz.
<i>H. c. occidentalis</i> Ridgw.	***	*	*	*	*	R. coast of w. Mex.
<i>H. redivivus</i> (Gamb.)	****	**	**	**	***	R. Pacific coast of Cal. & L. Cal.
<i>H. lecontei</i> (Lawr.)	****	*	**	*	**	R. vall. of Colorado and Gila rivers.
<i>H. crissalis</i> (Henry)	****	*	*	**	**	R. N. M., Ariz., s. Utah, s. Cal., n. L. Cal.
<i>Heleodytes brunneicapillus</i> (Lafr.)	***	*	*	*	*	R. s. w. border of U. S.
<i>H. affinis</i> (Xantus)	**	*	*	*	*	R. s. L. Cal.
<i>Salpinctes obsoletus</i> (Say)	****	*	*	****	**	R. arid distr. of w. U. S. s. to Guatemala.
<i>S. guadeloupensis</i> Ridgw.	*	*	**	*	***	R. Guadelupe I.
<i>Catherpes mexicanus</i> (Swains.)	****	****	*	*	*	R. Mex., s. Tex.
<i>C. m. conspersus</i> Ridgw.	****	*	**	*	****	R. s. w. U. S. n. to Ore.
<i>Thryothorus ludovicianus</i> (Lath.)	***	**	****	**	****	M. n. e. Mex. & e. U. S. to 40° lat.
<i>T. l. miamensis</i> Ridgw.	**	**	**	*	***	R. s. e. Fla.
<i>T. albinucha</i> (Cabot)	*	*	*	*	*	R. Yucatan & Guatemala.
<i>T. bewickii</i> (Aud.)	**	*	****	**	**	M. e. U. S. n. to 40° lat., w. to Gt. Plains.
<i>T. b. spilurus</i> (Vig.)	***	*	****	**	**	R. coast from w. Mex. to B. C.
<i>T. b. bairdi</i> Salv. & Godm.	****	**	**	***	****	R. table-lands of Mex. to Kan.
<i>T. brevicaudus</i> Ridgw.	*	*	*	*	*	R. Guadelupe I.
<i>T. felix</i> Scl.	*	**	**	**	*	R. w. Mex.
<i>T. lawrenci</i> (Ridgw.)	*	*	*	*	*	R. Tres Marias Is.
<i>T. maculipectus</i> Lafr.	**	*	*	*	*	R. s. Mex.
<i>T. m. umbrinus</i> Ridgw.	*	*	*	*	*	R. Guatemala.
<i>T. m. cannohrunneus</i> Ridgw.	*	*	*	*	*	R. Yucatan.
<i>Troglodytes insularis</i> Baird	*	*	***	*	*	R. Socorro I.
<i>T. beani</i> Ridgw.	**	*	*	*	*	R. Cozumel I.
<i>T. ædon</i> (Vieill.)	**	**	***	****	****	M. e. U. S. & Canada, w. to Miss. vall.
<i>T. a. parkmanii</i> (Aud.)	****	**	***	****	**	M. w. U. S. s. to Vera Cruz.
<i>T. intermedius</i> Cab.	**	*	**	*	**	R. s. Mex. to Costa Rica.
<i>T. brunneicollis</i> Scl.	*	*	*	*	*	R. s. e. Mex.
<i>T. hiemalis</i> Vieill.	***	**	**	***	****	M. n. e. U. S. northward.
<i>T. h. pacificus</i> Baird	**	*	*	*	**	M. coast from s. Cal. to Sitka.
<i>T. alascensis</i> Baird	**	*	*	*	**	R. Aleutian & Prybilof Is.
<i>Cistothorus stellaris</i> (Licht.)	***	**	*	****	*	M. e. U. S. w. to Gt. Plains.
<i>C. polyglottus</i> Vieill.	**	*	*	*	****	R. e. trop. A. from Mex. to Brazil.
<i>C. palustris</i> (Wils.)	****	***	**	****	***	M. e. U. S. & Brit. Prov.
<i>C. p. paludicola</i> Baird	**	**	**	****	**	M. w. U. S. to Rocky Mts.

TABLE XI. VARIATION IN THE PARIDÆ.

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
<i>Sitta carolinensis</i> Lath.	**	*	*	***	**	R. e. U. S. & Brit. Prov.
<i>S. c. aculeata</i> (Cass.)	***	**	*	****	***	R. w. U. S. into Mex.
<i>S. canadensis</i> Linn.		*				M. chiefly n. of U. S.
<i>S. pusilla</i> Lath.	****			**		R. s. Atlantic & Gulf St.
<i>S. pygmæa</i> Vig.	*			***		R. w. U. S. to Mex., e. to Rocky Mts.
<i>Parus atricristatus</i> Cass.		**		***		R. e. Mex. to s. Tex.
<i>P. a. castaneifrons</i> Sennett		*		*		R. e. Tex.
<i>P. inornatus</i> Gamb.	*	*	*	***		R. coast from s. Cal. to Ore.
<i>P. i. cinerascens</i> Ridgw.	****		*	*		R. s. L. Cal.
<i>P. i. griseus</i> Ridgw.	****	*	**	*	**	R. Rocky Mt. distr. of U. S.
<i>P. wollweberi</i> (Bonap.)		*		**	**	R. highlands of Mex. to s. Ariz.
<i>P. gambeli</i> Ridgw.		**		***	*	R. mts. of w. U. S.
<i>P. meridionalis</i> Scl.		*	*	**	*	R. highlands of Mex. n. to s. Ariz.
<i>P. carolinensis</i> Aud.		*	***	*	***	R. e. U. S. s. of 40° lat.
<i>P. atricapillus</i> (Linn.)		*	*	****	*	M. e. N. A. n. of 40° lat.
<i>P. a. occidentalis</i> (Baird)	***	*	***	**		R. n. w. coast distr. of U. S.
<i>P. a. septentrionalis</i> (Harris)	*	***	****	***		R. Rocky Mt. distr. from N. M. to Alaska.
<i>P. cinctus obtectus</i> (Cab.)	*	*	*	*	*	R. e. Siberia & n. Alaska.
<i>P. hudsonicus</i> Forst.	**	**	***	****		R. ? n. N. A. e. of Rocky Mts.
<i>P. rufescens</i> Towns.	***	***	**	***		R. coast from Ore. to s. Alaska.
<i>P. r. neglecta</i> Ridgw.	**	***		**		R. coast of Cal.
<i>Psaltiparus minimus californicus</i> Ridgw.	**	**	**	***		R. Cal.
<i>P. m. grindæ</i> (Beld.)	*		*	*		R. s. L. Cal.
<i>P. plumbeus</i> Baird	*	**	**	*		R. Rocky Mts. from Col. to s. Ariz.
<i>P. melanotis</i> (Hartl.)	*	*		*		R. highlands of Mex. & Guatemala.
<i>Auriparus flaviceps</i> (Sund.)	**		***	***		R. arid distr. of n. Mex. & contiguous U. S.
<i>Chamæa fasciata</i> Gamb.	**	***	*	***		R. coast of Cal.
<i>C. f. henshawi</i> Ridgw.	**	**	**	***		R. int. of Cal.

TABLE XII. VARIATION IN THE TURDIDÆ.

<i>Myadestes townsendi</i> (Aud.)		**		*	*	M. mts. of w. U. S. to B. C.
<i>M. obscurus</i> Lafr.	**	*	*	*		R. highlands of e. Mex. & Guatemala.
<i>M. o. occidentalis</i> Stejn	*	*	*			R. c. & w. Mex.
<i>M. o. insularis</i> Stejn		*		*		R. Tres Marias Is.
<i>M. unicolor</i> Scl.		*	*	*		R. highlands of s. Mex. & Guatemala.
<i>Turdus mustelinus</i> Gmel.	****	*	*	**	**	M. e. U. S. to Mass.
<i>T. fuscescens</i> Steph.	***	**	***	****	****	E. M. e. N. A. between 40° & 50° lat.

TABLE XII. — *Continued.*

SPECIES.	CULMEN.	WING.	TARSUS.	WHOLE LENGTH.	TAIL.	MIGRATION. BREEDING AREA.
T. f. salicicolus (Ridgw.)	*	**	**	**	***	M. Rocky Mts.
T. aliciae Baird	****	***	***	**	***	E. M. Labr. to Arctic coast.
T. a. bicknelli (Ridgw.)	*	**	**	***	*	E. M. Catskill Mts. to Nova Scotia.
T. ustulatus (Nutt.)	****	**	*	**	***	E. M. Pacific coast n. to Sitka.
T. u. swainsonii (Cab.)	**	*	**	***	**	E. M. e. N. A. n. of U. S.
T. aonalaschkæ Gmel.	***	***	*	***	***	M. coast from Cal. to Kodiak.
T. a. auduboni (Baird)	**	***	**	**	***	M. Rocky Mts.
T. a. pallasii (Cab.)	****	**	**	***	****	M. n. e. U. S. northward.
T. iliacus Linn.	**	*	*	**	***	M. n. Eurasia.
Merula migratoria (Linn.)	*	**	*	**	*	M. e. N. A. n. to Hudson Bay & Alaska.
M. m. propinqua Ridgw.	**	*	***	**	****	M. w. U. S. n. to B. C., e. to Rocky Mts.
M. confinis (Baird)	**	***	*	*	*	R. s. L. Cal.
M. flavirostris Swains.	***	***	*	*	***	R. w. & s. Mex.
M. graysoni Ridgw.	*	*	*	*	*	R. Tres Marias Is.
Hesperocichla nævia (Gmel.)	*	*	*	**	*	M. w. N. A. n. to Behring Strait.
Cyanecula suecica (Linn.)	*	*	*	*	*	M. n. Eurasia.
C. wolfii Brehm	**	*	**	*	*	M. c. Europe.
Saxicola œnanthe (Linn.)	**	**	****	***	****	M. n. portions of N. Hemisphere.
Sialia sialis (Linn.)	♂	*	*	*	****	M. e. U. S. w. to Rocky Mts.
	♀	*	*	*	*	
S. s. azurea (Baird)	♂	*	*	*	*	R. highlands of Mex. & s. Ariz.
S. s. guatemalæ Ridgw.	♂	*	*	*	*	R. highlands of Honduras & Guatemala.
	♀	*	*	*	*	
S. mexicana Swains.	♂	**	**	**	*	R. w. U. S. n. to B. C., e. to Rocky Mts.
S. arctoa (Swains.)	♂	*	*	****	*	M. Rocky Mts. n. to Gt. Slave Lake.
	♀	*	*	*	*	

The following table (XIII) represents the percentage of species and subspecies examined, of fourteen families, evincing variation of at least 1.5% in two dimensions. Only such species and subspecies enter into the computation for which Ridgway's measurements express variation in at least three of the five dimensions; and the percentages have been deduced separately for non-migratory, migratory, and extensively migratory species and subspecies.

TABLE XIII.

FAMILY.	NON-MIGRATORY SPECIES.		MIGRATORY SPECIES.		EXTENSIVELY MIGRATORY SPECIES.	
	Number examined.	Per cent.	Number examined.	Per cent.	Number examined.	Per cent.
Anatidæ	3	33.3	31	35.4	17	41.1
Rallidæ	3	66.6	8	25	5	80
Scolopacidæ	1	100	21	42.8	22	50
Falconidæ	25	32	10	40	4	25
Picidæ	36	19.4	8	25	1	0
Tyrannidæ	29	10.3	13	23	8	25
Corvidæ	42	30.9	0		0	
Icteridæ	17	17.6	5	40	1	0
Fringillidæ	59	23.7	64	26.5	15	40
Vireonidæ	9	0	5	0	7	14.2
Mniotiltidæ	14	0	19	26.3	24	33.3
Troglodytidæ	37	21.6	11	63.6	1	100
Paridæ	22	36.3	1	0	0	
Turdidæ	9	11.1	15	33.3	5	60

The following table (XIV) represents the percentage of species and subspecies examined, of nine families, which exhibit an amount of variation of at least 1.5% in two out of at least three dimensions examined. These percentages are computed separately: for (1) distinct species (*i.e.* without geographical races), and (2) geographical races. In this computation necessarily *Melospiza fasciata*, *e.g.*, is considered a race, just as is *M. f. montana*.

TABLE XIV.

FAMILY.	DISTINCT SPECIES.		GEOGRAPHICAL RACES.	
	Number examined.	Per cent.	Number examined.	Per cent.
Anatidæ	56	25	12	33.3
Rallidæ	12	50	2	100
Falconidæ	26	34.6	13	30.7
Picidæ	19	15.7	26	19.2
Corvidæ	17	23.5	24	33.3
Fringillidæ	60	21.6	74	32.4
Troglodytidæ	31	22.5	19	47.3
Paridæ	11	27.2	17	29.4
Turdidæ	14	14.2	16	43.7

The following four tables (XV–XVIII) give respectively the percentage of those species and subspecies examined, in each family enumerated, showing an amount of individual variation of at least 2% in the particular dimension (culmen, wing, tarsus, or whole length). Only such families are computed, for which Ridgway's data express individual variation in the particular dimension for at least ten species.

TABLE XV.

Comparative tabulation of percentage of species examined, with variation in the length of the *culmen* of at least 2%.

FAMILY.	Number of species examined.	0%	.5%	1%	10%	20%	30%	40%	50%
Vireonidæ	18				11.1				
Tyrannidæ	43				13.9				
Mniotiltidæ	14				14.2				
Icteridæ	31				16.1				
Corvidæ	35				16.6				
Turdidæ	24				16.6				
Falconidæ	64				17.1				
Fringillidæ	109				17.4				
Troglodytidæ	47					23.4			
Picidæ	42					28.5			
Rallidæ	16						37.5		
Paridæ	10							40	

TABLE XVI.

Comparative tabulation of percentage of species examined, with variation in the length of the *wing* of at least 2%.

FAMILY.	Number of species examined.	0%	.5%	1%	10%	20%	30%	40%	50%
Vireonidæ	26	0							
Mniotiltidæ	71	0							
Paridæ	26	0							
Turdidæ	32	0							
Icteridæ	43	0							
Corvidæ	42	0							
Tyrannidæ	63	0							
Fringillidæ	181		.55						
Troglodytidæ	49			2					
Picidæ	48			4.1					
Falconidæ	89			7.8					
Rallidæ	16				12.5				

TABLE XVII.

Comparative tabulation of percentage of species examined, with variation in the length of the *tarsus* of at least 2%.

FAMILY.	Number of species examined.	0%	.5%	1%	10%	20%	30%	40%	50%
Vireonidæ	14	0							
Paridæ	20	0							
Tyrannidæ	34	0							
Icteridæ	23	0							
Fringillidæ	99			2					
Corvidæ	34			2.9					
Turdidæ	25			4					
Mniotiltidæ	18			5.5					
Rallidæ	16			6.2					
Troglodytidæ	46			6.5					
Falconidæ	78				19.2				

TABLE XVIII.

Comparative tabulation of percentage of species examined, with variation in the whole length of at least 2%.¹

FAMILY.	Number of species examined.	0%	.5%	1%	10%	20%	30%	40%	50%
Picidæ	31			3.2					
Tyrannidæ	28			7.1					
Icteridæ	39				10.2				
Fringillidæ	118				11.8				
Vireonidæ	16				12.5				
Falconidæ	53				13.2				
Paridæ	22				13.6				
Turdidæ	20				15				
Corvidæ	34				17.6				
Troglodytidæ	34					20.5			
Mniotiltidæ	53					28.3			
Rallidæ	12							41.6	

¹ Whenever a species has been considered in the calculations of Tables XV-XVIII, for which Ridgway gives the extremes of variation in a particular dimension separately for the sexes, I have computed and added the percentage of each sex as an equivalent to that of a species. This method would hardly impede the general accuracy of the percentages.

TABLE XIX.

Exhibiting variation in relation to sex, the figures in the vertical columns referring to the number of species and subspecies examined.

FAMILY.	♂		♀	
	Larger, with greatest variation.	Smaller, with greatest variation.	Larger, with greatest variation.	Smaller, with greatest variation.
<i>Culmen.</i>				
Falconidæ	0	10	13	0
Trochilidæ	1	6	0	1
Icteridæ	6	0	0	4
Fringillidæ	7	3	0	1
<i>Wing.</i>				
Falconidæ	0	19	16	0
Trochilidæ	2	5	1	0
Icteridæ	8	0	0	5
Fringillidæ	13	0	1	9
<i>Tarsus.</i>				
Falconidæ	2	18	8	1
Trochilidæ				
Icteridæ	3	0	0	2
Fringillidæ	6	0	1	6
<i>Whole Length.</i>				
Falconidæ	0	11	9	0
Trochilidæ	0	5	2	0
Icteridæ	5	0	0	8
Fringillidæ	1	0	0	4
Totals,	54	77	51	41
	= 131		= 92	

TABLE XX.

Differing from the preceding table in so far, that in addition to the four families therein enumerated, all species and subspecies of other families are computed, for which Ridgway has given individual extremes of measurements for each sex.

DIMENSIONS.	♂		♀	
	Larger, with greatest variation.	Smaller, with greatest variation.	Larger, with greatest variation.	Smaller, with greatest variation.
Culmen	15	19	15	7
Wing	41	26	20	21
Tarsus	11	20	9	10
Whole length	16	17	12	14
Totals,	83	82	56	52
	= 165		= 108	

C. *Direct Inferences from the Tabulated Data.*

(a) It is the rule that, in genera comprising more than one species, those species which inhabit small or insular breeding areas do not evince as much individual variation in the dimensions as do species with more extensive and diversified breeding areas. This fact becomes at once apparent after a study of the Tables I–XII, when a comparison is made between species inhabiting small islands, or other restricted districts, and those which have a much wider distribution. But few exceptions are to be found to this rule.

(b) It is the rule that species with geographical races, when the latter differ from one another in one or more dimensions, evince a greater amount of individual variation than do species which are not divided into such races, provided that the breeding area is approximately equal in extent and diversification in both cases. Thus of the nine families tabulated in Table XIV, in all, with the single exception of the *Falconidæ*, a greater percentage of geographical races evince variation to the amount of 1.5% in two dimensions, than of species which are not split into races. If, however, the geographical races of a species differ from one another mainly in color (as *e.g.* those of *Cardinalis cardinalis*), and less or not at all in dimensions, then as a rule they do not evince a greater amount of variation in the dimensions than do species without geographical races.¹ In Table XIV, further, the percentages in favor of geographical races may still be increased, when we consider that many species which are as yet regarded as distinct, may in the future be classed by ornithologists as subspecies,—resulting in a subtraction from the left hand column of percentages, and an addition to that on the right.

(c) It is the rule, subject to the preceding two “laws,” that migratory species evince a greater amount of individual varia-

¹ Though I have not investigated in birds the facts of individual color variation,—a kind of variation of which it is obviously difficult to determine the amount, I would be inclined to conclude, *a priori*, that the races of a species differing from one another mainly in color would present a greater amount of color variation than would species which are not divisible into color races, other factors being equal in both cases.

tion than do non-migratory species ; and species which undertake extensive migrations, a greater amount than species which make migrations of less magnitude (Table XIII). This fact is conformable with our "law" *a*, since migratory species have as a rule more extensive breeding areas than have non-migratory species. Thus of the 110 species which undertake extensive migrations, entering into the computation of table XIII, 104 (94.5% inhabit breeding areas of comparatively great extent, while but 6 (5.4%) inhabit small areas.¹

(*d*) It is the rule, that males exhibit a greater amount of individual variation in the dimensions than do females of the same species or subspecies. For of the 223 computed measurements of both sexes in Table XIX, in 131 cases (58.7%) the males show the greater amount of variation, and in 92 cases (41.2%) the females, — a difference of about 17.5% in favor of the males ; and of the 273 measurements of both sexes computed in Table XX, in 165 cases (60.4%) the males show the greater amount of variation, and in 108 cases (39.5%) the females show the greater amount, — a difference of about 20.8% to the advantage of the males.

(*e*) It is the rule that there is less variation in the length of the wing than in the length of the culmen, tarsus, or whole bird. This fact becomes at once apparent, by comparing the "curve" of variation expressed in Table XVI with the "curves" of variations of the other three dimensions (Tables XV, XVII, XVIII).

We may now consider the support given by these five "laws" to the thesis, that continuing development is always accompanied by variability.

To recapitulate briefly : it follows from the data given, that the greatest amount of individual variation occurs, as a rule, in those species occupying the most extensive breeding areas ; that of two species occupying breeding areas approximately equivalent in extent, the one divided into geographical sub-

¹ These six species are *Ammodramus lecontei*, *Spizella monticola ochracea*, *Passerella iliaca unalaschcensis*, *Chen caerulescens*, *C. hyperborea*, *Turdus aliciae bicknelli* ; of these, only the first and third evince variation to the amount of 1.5% in two dimensions.

species evinces a greater amount of variation than the "stable" species ; and that species which undertake extensive migrations exhibit more individual variation, other factors being equal, than do species which do not migrate. Now in the first part of this second section, we have found that the presence of geographical races (subspecies) and migration are two criteria of continuing development. Therefore, the fact that the amount of variation is greater in migratory species, and in species which exhibit geographical races, than in non-migratory species, or than in species which present no geographical subspecies, is a sufficient proof for the assertion that continuing development is always associated with variability. In other words, individual variation is greater in amount in those species which we must consider under the influence of a continuing process of development than in those species which we must consider as being influenced by no process of development at all, or by a much less energetic development. And as we have found variation is as a rule more marked in migratory than in non-migratory species, and in extensively migratory than in less migratory species ; and further, that as a rule, greater individual variation is present in the several races of a species which exhibits a large number of races than in the races of a species exhibiting a smaller number, — therefore we must conclude that the amount of individual variation stands in a direct ratio to the activity and energy of the operating process of development. In short, the logical sequence from the facts given is plainly that the amount of individual variation stands in a direct ratio to the degree of complexity of the environmental forces which influence the organism. A species with an extensive breeding area, or one which meets with environmental changes in the course of its migrations, is more variable than a species with a restricted and little diversified breeding area, or than a non-migratory species, which does not come into contact with new environments.

The fact that the dimensions of birds are more variable in the males than in the females is interesting, as offering a parallel to the case in man, where, too, the males are more

variable.¹ Also in domesticated animals, the males are more variable (Darwin). Why the wing should be less variable than the other dimensions, is difficult of solution on any other ground, than that the wing has but one main function, while the tarsus and the bill are put to a diversity of uses, which would result in the two latter being more variable.

III. ON THE ORIGIN OF VARIATION.

The doctrines of Lamarck and Darwin teach that the organism is more or less adapted to its environment ; even Bateson (*l.c.*, p. 10) grants a certain amount of such adaptation, though in the main he militates against the assumption of any complete degree of adaptation. We may then start out from the assumption, for the correctness of which there is strong evidence, that it is a biological law for the organism to be more or less completely in correlation with its environment, in order to insure its existence. It is even permissible to go further, and assert that its chance of existence will stand in direct proportion to the degree of its adaption to the environment. (By the term environment, which is used here in its full sense, is meant the sum total of all the external forces acting upon the organism.) Therefore it is obvious that the chance of the organism's existence must depend upon its degree of correlation with the environment, or, in other words, with its readiness of response to the external conditions. This law being so reasonable, and so thoroughly in accord with our modern biological ideas, it is not necessary to enter into any further discussion of its plausibility.

For the purpose of advancing further deductions as to the primal cause of variation, we may proceed from the consideration of a species which, we have reasons to conclude, is, comparatively speaking, perfectly adapted to its environment. We

¹ It is not impossible, that in birds, as in man, the female may be more conservative and less progressive than the male, and passing a more (physiologically) monotonous existence than the latter, is less influenced by the struggle for existence, and accordingly is less variable structurally. This suggestion has, however, no more value than that of a mere comparison.

may take, for example, a species inhabiting a small island, which is comparatively uniform in character throughout its extent (in vegetation, etc.), and at all seasons of the year (climate, etc.). Such insular species are very numerous among land-inhabiting animals of tropical distribution. The environment influencing the insular species being then so uniform and unchanging in its action upon it, the species could more easily and quickly adapt itself to it, than if the environment were changeable. Taking then an insular species, which, we have reasons to suppose, has inhabited a particular island for a comparatively long period, — and there are usually certain criteria whereby we may judge whether its residence there has been of long duration, — we must suppose that it had time to become adapted to its environment; and if we have equally good reasons for supposing that the character of the environment itself has not changed, it must seem probable that its adaptation to the environment is comparatively perfect. Granting such a species, accordingly, to be closely adapted to its environment, let us consider what changes, if any, would occur in the organism, if the environment should change. And it may be remarked just here, that marked changes have been recorded by geologists and others in different districts, as is well known, such as a surface rising or sinkage, changes in vegetation due to a prolonged drought, the invasions of organisms strange to the region, destruction of life caused by epidemics, etc. And in fact, in many districts where the environment appears to the human sense to be practically unchanging, a slow and gradual change may nevertheless be taking place.

Now such an organism is adapted to its environment, at the same time that its various organs must necessarily be correlated to one another. By correlation of the organs is meant, as was explained in the introductory part of this paper, their mutual dependence upon, and concerted physiological action with, each other. Thus, in the case under question, we must treat together the two facts: (1) the adaptation of the organism to its environment, and (2) the physiological and morphological correlation of its organs. When a change of environment occurs, *i.e.* when consequently a new and different environment

commences to influence the organism, the latter cannot at first be adapted to this new environment, but must become, so to speak, out of touch with it. This change of environment must then influence the organism, primarily, by disturbing or interrupting the correlation of its organs. For even should the change of environment interrupt directly the physiological action of only one of the organs, this particular organ would no longer be capable of acting in concerted harmony with the other organs, and thus the correlation of the whole would be disturbed. For example, if the customary food supply become exhausted, so that the organism is compelled to seek nourishment of a different kind, not only in the immediate intestinal cells must a physiological (and consequent morphological) change ensue, but also the structure and function of each organ, indirectly dependent upon the intestine's action, must become modified. And, *pari passu*, if the external forces acting upon a sense-organ assume a different direction, not only must this particular organ become physiologically modified, but indirectly also the nervous system, and all organs in functional communication with the latter; or if a certain organ become diseased, its normal action must become to some extent impeded, which would exert an influence upon the functions of the other organs. In fact, whatever organ be directly influenced by a change in the environment, a modification of the functions and structure must result in those organs which are in correlation with it.

Accordingly, after a change in the environment, a temporary disturbance of the correlation of the organs must result. When we speak in this way of an "interruption" or of a "disturbance" of the correlation, we mean that the mutual dependence of the several organs upon one another is reduced, so that they become to an inverse extent independent of one another. Now I consider that the origin of variation is to be found in this condition of temporary independence of the organs, which independence is caused by change of environment, resulting in the partial interruption of the organs' correlation. For each organ, after the disturbance of such mutual correlation, becomes more *autodynamic*, — more independent of the restraining influences of

the others, and consequently its own forces of growth and action may operate more freely and to greater extent than was possible in the previous condition, when it was held in check by the state of correlation with the other organs. In other words, after any change of the environment, that is, after any consequent interruption of the correlation of the organs, each organ becomes temporarily more autodynamic than it was before, and the comparatively independent action of its vital forces may result in the production of abnormalities, which are known as variations. To what extremes these variations may go in amount or extent will be considered in the next section.

The question arises at this point in the argument : why, when through the interruption of the correlation of the organs a certain degree of temporary independence of each of the latter ensues, should any of the particular organs exert this independence in the production of structural variations? Now we have seen that the chances of existence of an organism stand in a direct proportion to the degree of its adaptation to the environment ; and further, that a complete correlation of its organs is necessary before a perfect adaptation to its environment is possible, thereby its chance of existence depending upon the degree of correlation of its organs. Accordingly, when the correlation of the organs has been disturbed by a change in the environment, it has but a small chance of existence until this correlation is restored. This deduction can hardly be questioned, because it is difficult to conceive of an organism existing when the correlation of its organs is greatly impaired. It follows, therefore, that the organism must attempt, in its fight for life, to restore this correlation, which is obviously a step necessary for its becoming adapted to the new environment. Plainly, then, after the correlation of the organs has become more or less interrupted, the several organs would exert their degree of temporary independence of one another in such a manner as to restore the correlation. Bearing this point in mind, and remembering in this connection the previous assumptions of our argument, we conclude : that *organic structural variations are the morphological results of physiological exertions on the part of the organism, to restore that complete correlation*

of its organs which had been disturbed by a change of environment. Even in the case of a disease attacking an organism, may we not regard any abnormal growth produced by the afflicted organism itself, to be the structural result of vital processes in the organism striving to restore the correlation of its organs? Indeed if we consider, which we must in view of the facts at hand, that correlation of the organs is a physiological necessity for the existence of an organism, then, if this correlation be disturbed by a change of environment, we are logically forced to conclude that the organism must make the attempt to restore this correlation, and that structural variations would be the result of such a physiological exertion.

A similar explanation of the origin of structural variations can be reached also from another standpoint : when a change of environment disturbs the correlation of the organs, the correlation when restored must differ to some extent from the previous state of correlation, since the new environment exerts a different influence upon the functional activity of the organs. But, as a different state of correlation cannot be conceived as existing in the same structural unity, certain changes of structure are necessarily involved, and these we term "variations."

The statistics given in this paper on variation in birds show that species with restricted breeding ranges and which are non-migratory in habit are, as a rule, much less variable with regard to dimensions — other factors being approximately equal — than are species occupying more extensive and more diversified areas, and which undertake periodical migrations of considerable magnitude. We are justified in concluding from these facts, that as a rule the amount of variation stands in direct ratio to the degree of environmental diversity of the inhabited area, *i.e.* to the amount of change of environment which influences the organism. And, as the degree of interruption of the correlation of the organs must be in direct proportion to the amount of change in the environment, the greater variation in those species of extended and diversified habitats is easily and solely explainable on our deduction, that the greater change in the environment should cause greater physiological independence between the several organs ; and therefore the structural changes ensu-

ing from the physiological exertions to restore the correlation, should be greater than in species of comparatively restricted and uniform habitats which experience neither so many nor so great changes of environment.

Recapitulation. — Organic variation owes its origin indirectly to change of environment. For it is necessary for the existence of an organism that its organs be correlated physiologically and (consequently) morphologically, and no adaptation to its environment, a factor which is also necessary for its existence, can be brought about until the organs become correlated. Now when a change occurs in the environment, this change checks the normal action of one or more of the organs, and influences indirectly the others, so that the correlation of the organs becomes temporarily disturbed or interrupted. The degree of disturbance in the correlation of the organs, probably stands in a direct ratio to the amount of change in the environment ; and according to the statistics given above, the amount of variation certainly stands, as a rule, in direct proportion to the complexity of the environment. Naturally, when an interruption of the correlation occurs, the several organs become to such a degree independent of one another as is the extent of its disturbance : the greater the disturbance of the correlation of the organs, the more autodynamic the individual organs become. In order to adapt itself to the new environment, the organism must first restore the correlation of its organs. Now the several organs, being no longer held in strict restraint by the agency of a complete correlation, make use of their temporary degree of physiological independence in order to restore this correlation. Any structural changes resulting from the exertions of the comparatively unrestrained (independent or autodynamic) physiological forces of the organs, to restore their correlation, are organic variations.

IV. ON VARIATION AS A CRITERION OF DEVELOPMENT.

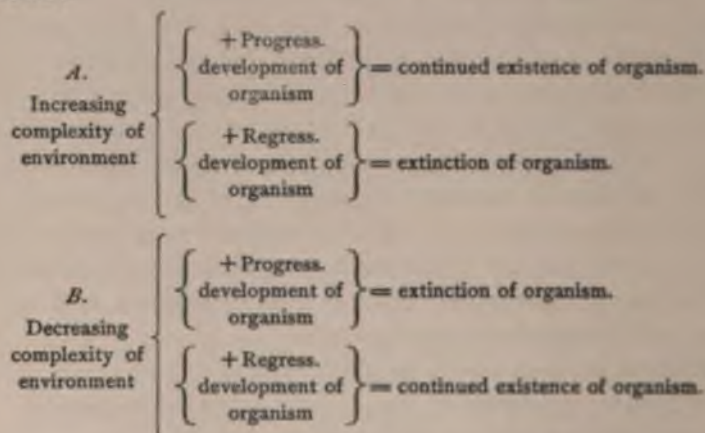
In the preceding pages I have tried to analyze briefly the processes of progressive and regressive development, and from a study of the facts of variation in birds, which show that the

amount of variation stands in a direct proportion to the amount of change in the environment, to advance a theory of the origin of variation, — as due directly to the physiological exertions on the part of the organism, to restore the correlation of the organs which had been disturbed by a change of environment. This theory is sustained by the facts given here, and if it be corroborated by future studies, we may use it as a standpoint from which to review the phenomena of organic variation, as offering criteria for the study of the processes of development.

The question often recurs to the biologist engaged in comparative anatomical investigations, why in a certain species a particular organ should be structurally variable, which is eminently stable in allied species. It has, thus far, been the method of the biologist to attempt an explanation of this variability by reasons derived from his assumptions as to the phylogenetic origin of the group, and of the different forms comprising it. But may we not, conversely, acquire some understanding of the hitherto unknown, or but hypothetically conjectured, development and homologies of an organ, by starting out from the facts of the phenomena of variation themselves? This is a line of research inaugurated by Bateson, and which may in time afford important results.

First of all, it is well to recall to mind the two factors necessary for the existence of the organism: (1) its adaptation to the environment, and (2) the correlation of its organs. When the environmental forces become more complex in their action, the intimacy of the correlation of the organs being more or less in a direct proportion to the degree of complexity of these external forces, as will be shown later, — then the structural development occasioned by such a change of environment must be a progressive one, if the organism would maintain its adaptation to the environment. But if, on the other hand, the change of environment is tending toward a simplification of the previously complex action of the environmental forces, then the organism must undergo a regressive structural development in order to remain in adaptation to the environment. In other words, if the environment is becoming more complex, the structure of the organism must also become more complex in order to insure its

adaptation ; while if the environment is becoming less complex, then the structure of the organism must become less intricate. For if the environmental action become more complex while the structure of the organism remains as it was, or becomes more simple, then the latter can no longer continue to exist ;¹ or if the environment become less complex in its action while the organism's structure either remains as it was or becomes more complicated, its extinction must be brought about. These relations of changes in the environment to changes in the structure of the organism, with reference to their effect on the existence of the latter, may be graphically represented as follows :



Accordingly, when the phenomena of variation are more clearly understood, and the direction and quantitative amount of change in the environment can be determined, then it will be possible to predict the future of a given organism.

Now variation, as I have tried to explain it, expresses a want of correlation between the several organs ; and such an interruption of the correlation can be caused only by the agency of a change of environment. Accordingly, it is permissible to state of a variable organ that it is not in complete correlation with its fellow organs ; and consequently, further, that some change is occurring, or has taken place, in the environment.

¹ A possible example of such a case is that afforded by the now extinct group of Ammonites.

Therefore, in order to explain the presence of variation in a certain species of a group not present in the same organ of closely allied species, we must compare the conditions of the environment of the one with those of the others. Thus, regarded from this standpoint alone, when variation is perceptible in organ x of species A , but not so in organ x of a closely related species B , we may conclude that organ x of species A is being influenced by some change of environment which is not affecting the corresponding organ x of species B . May we not consider that the particular organ in A is commencing to develop in a new direction, while the organ in B is remaining unchanged? By this would be merely shown, however, that whenever variation is noticeable, the organ evincing it is tending towards an ultimate structural modification, due to the fact of a change of environment already taking, or having taken, place.

What light can the phenomena of variation throw upon the phylogeny of organisms? I consider that it may be possible to decide, with a certain degree of certainty, whether a given species is developing progressively or regressively at the present time, and whether in the near past it has progressed or degenerated; by basing our conclusions as to its course of development, present and past, upon the direction and degree in which the variation appears. This may seem to be a bold assumption, but if the views expressed in this paper upon the nature and origin of variation be probable, we may yet learn that the study of variation may furnish valuable criteria for estimating the facts of phylogeny. As Bateson observes (*l.c.*, p. 6), two criteria of phylogeny upon which much confidence is misplaced, namely the ontogeny and the direct study of adaptation, are by no means infallible; so that to-day we have only the criterion of the facts of palæontology, — a criterion which Bateson fails to mention, with which we may feel ourselves secure. And this being the case, we should gladly avail ourselves of a further criterion, namely, the phenomena of variation.

In comparing the antagonistic states, progressive and regressive development (*cf.* Section I), it was found that progressive development leads towards a more complicated structural modi-

fication, and regressive development to a structural simplification of the organism. Now, as is generally conceded, species are maintained in the struggle for existence by the preservation of favorable individual variations, *i.e.* (to my mind), variations which are favorable, in as much as they tend to produce a complete correlation of the organs; and all such structural variations are necessarily either more complex or more simple than the normal. The preservation of favorable individual variations which are more complex would result in the production of a more highly differentiated species; and, on the other hand, the preservation of those which are less complex would result in the formation of a morphologically less differentiated species. Accordingly, we must first determine whether the variations are above or below the normal, — more complex or more simple. For if the variations are more complex, then if they should be preserved a more highly organized species would be evolved; and if they are structurally simpler, and should be preserved, a less highly organized. Similarly, judging from the palæontological remains of a series of individuals of a now extinct species, it might be possible, after a careful investigation into the nature and amount of individual variations exhibited by them, to conclude whether a more highly or a less highly organized form, if any, had been produced. Thus we should expect, that a given species *A* occurring in the Liassic beds, presenting individual variations (osteological, *e.g.*) more complex than the normal, would be represented in the Triassic by a more highly differentiated species, if by any.

But the study of variation, thus far considered, gives us criteria for only the future development of the species, so that it remains necessary to seek criteria from the phenomena of variation for the past phylogenetic stages.

And, firstly, it is desirable to determine as far as possible what limits there are to individual variation. It seems to be well ascertained that there is a limit to such variation, but where that limit may be placed for a given organism, or what organic law fixes it, is very difficult of experimental proof. Since we find the amount of variation to be in direct proportion to the amount of change in the environment, the question is,

in other words, how great a change of environment the organism can withstand without serious injury. Now from a number of carefully made experiments, as noticeably the recent observations of Davenport and Castle,¹ we find that an organism which would be killed by a sudden change of temperature of 10° C., may become acclimated to that amount of increase in temperature if the change is made gradually. This fact proves that an organism can withstand only a certain maximum amount of sudden change of environment, while if the change be greater than this maximum amount, death ensues. Still another fact is important in this connection : lowly organized forms are, as a rule, more *widerstandsfähig* than highly organized forms ; it suffices, as an example, to call to mind the great changes of temperature which are not injurious to certain disease germs and swarm-spores, but which would cause the sudden death of a worm or mammal. From these facts we may conclude : (1) that as a given organism can withstand only a certain maximum amount of change in the environment, and since the amount of variation stands in a direct ratio to the amount of change in the environment, therefore the organism can produce only a certain maximum amount of variation ; and (2), since lowly organized forms can withstand greater changes of environment, as a rule, than can more highly organized forms, that the former can, as a rule, produce a greater amount of variation than the latter can. And these facts coincide perfectly with the general physiological law that the more differentiated the organs become structurally, the more intimate and complex becomes their correlation ; for more highly differentiated organisms, with a more complex correlation of their organs, are unable to produce variations to the same amount as can more lowly organized forms which have a less intimate correlation of their organs. Further, the correlation of the organs in lowly organized forms, being less complex, can be restored sooner after a change of environment than the correlation can be restored in more highly differentiated forms after the same amount of change. Thus we find that the amount of variation depends upon the degree

¹ "On the Acclimatization of Organisms to High Temperatures," Arch. f. Entwicklungsmech. d. Organismen, Bd. II, 1895.

of change in the environment, and upon the degree of differentiation of the organism ; but that a certain maximum amount of variation cannot be exceeded by the organism, and this amount seems to differ for different organisms. Variations must continue to be produced until the correlation of the organs is fully restored, when the restraint exerted by this acquired correlation upon the physiological processes of the several organs, would prohibit the production of further variations. Therefore, if the variations continue to be produced through a long period of time, we must conclude that the correlation of the organs has been greatly disturbed, which is equivalent to saying that a comparatively great change has occurred in the environment. If, however, but one considerable change has occurred in the environment and the latter remains thereafter unchanged, then the longer the period of time is which has elapsed since this change, the nearer at hand will be the restoration of the correlation of the organs, and consequently the less will be the amount of variation. This will serve further to elucidate the deduction made in a former paper¹ of mine (p. 483) : that "the amount of variability above or below a given mean will stand in inverse ratio to the length of time in which the development (progressive or regressive) has acted upon the given organ." If the change of environment be comparatively slight, the restoration of the correlation of the organs might be fulfilled in a single generation ; but if the change had been more marked, this correlation might not be restored until after the lapse of a large number of generations, during which time the production of variations would continue, though their amount would decrease as the time became longer.

Here, then, the phenomena of variation may furnish us with a criterion for deducing, to some extent at least, the previous conditions of existence, if not also the phylogenetic stages of some organisms. For we may briefly consider, *e.g.* the freshwater Nemerteans, which are undoubtedly of marine origin. In studying the comparative anatomy of this group of worms, I was struck by the fact that while the nearest marine allies of

¹ "The Derivation of the Freshwater and Land Nemerteans, etc.," JOURNAL OF MORPHOLOGY, XI, 2, 1895.

these freshwater species possess almost invariably four large eyes, the freshwater forms, on the contrary, have a larger number of eyes, varying from four to eight, which are also smaller than those of the marine species. How is this variability in the number of eyes of the freshwater forms to be explained? Now, variability is engendered, in our view, indirectly by change of environment; and we know that the species in question have changed their environment by migrating from bodies of salt water into freshwater rivers and lakes. (Or, in certain cases, they are inhabitants of lakes which were originally of marine character, but have become fresh.) The number of the eyes of the marine species being very stable, we conclude: (1) that the correlation of the organs in the marine forms is comparatively complete, and that, therefore, they are well adapted to their environment; and (2) that no variability being perceptible, they are neither at present giving rise to new species, nor are they themselves of recent origin. On the other hand, the eyes of the freshwater forms being very variable in number, we conclude for these: (1) that the correlation of their organs is not perfect, and hence that they are not fully adapted to their environment; (2) that this variability must have been caused by a change of environment within a comparatively recent period of time, since the variability is still continuing; and (3), that as the numerical variation of the eyes is above the normal four (the number 6-8 being in fact more frequent than 4 or 5), the species is tending to evolve a form with a greater number of eyes than its ancestors possessed (progressive numerical development in relation to the eyes).

A similar case occurs to me in regard to the individual variation in number in the rectrices (stiff tail-feathers), of certain North American species of grouse. Table XXI (placed at the end of the paper) expresses this variation for *Centrocercus urophasianus*, and for *Dendrapagus obscurus* with its two geographical races.¹ According to Mr. Clark's observations, these are the only North American species of grouse evincing such variation; the number of rectrices being stable in *Den-*

¹ I am indebted to the kindness of my friend Mr. Hubert Lyman Clark, of Baltimore, for the communication of the facts embodied in Table XXI.

drapagus canadensis, *D. franklini*, and in *Bonasa*, *Lagopus*, *Tympanuchus*, and *Pediæcetes*. Now it is interesting that the subgenus *Dendrapagus* of the genus *Dendrapagus* is limited to North America, and its species are variable in regard to the number of the rectrices; while such variation apparently does not occur in subgenus *Canace* of the same genus, which inhabits both Eurasia and North America. Of the other genera, *Bonasa* and *Lagopus* have the same geographical distribution as *Canace*, while *Tympanuchus*, *Centrocercus*, and *Pediæcetes* are limited to North America. Those individuals evincing this numerical variation of the rectrices must have been influenced by a considerable change of environment, since the variability has continued through a large number of generations. Now we can hardly suppose that this change of environment has occurred within the areas occupied by these variable species in North America, since in that case we must suppose that the other species occupying the same areas must have become similarly modified. Accordingly the species of *Dendrapagus* which presents this variation must be of comparatively recent occurrence within its present habitat; and its variability would be caused by having changed its former habitat within a comparatively recent period, by migrating from its former area (Eurasia?) to its present geographical position in America. On the other hand, the non-variable North American species of this genus (namely *canadensis* and *franklini*) must be regarded either as not having experienced such a change of environment, or as having experienced it in a much remoter period, having in the meanwhile restored the correlation of their organs, *i.e.* adapted themselves to the new environment. Regarded from this standpoint, the variation in the number of the rectrices may serve to elucidate the origin and present distribution of the *Tetraonidæ*.

These considerations are offered as mere suggestions for explaining how variation can serve as a criterion of development. We have found that the data of variation offer a certain mean for determining whether the development is progressive or regressive, depending upon the fact whether the variations are above or below the normal. When the phenomena of varia-

tion are better understood, for this study is at present nearly virgin ground, we will probably be enabled to deduce from it criteria, which will offer certain and valuable aid in the study of phylogeny. And another important line of investigation must become the study of the environment, and of the changes of the latter in their influence upon the organism. A considerable number of experiments have recently been made in this line upon the ontogenetic stages of organisms, and a smaller number upon the adult organism; and a careful analysis of the results of such experiments may give valuable aid in the discrimination of methods for the study of variation.

A final word in regard to *regressive development*. It is as yet an undecided question whether regressive development (or the action of Natural Selection during this mode of development) can result in the total disappearance of an organ, or whether a structural rudiment must remain. According to our conclusions upon the nature of variation, the latter would be the more probable view, and for the following reasons. For the action of a regressive development, the occurrence of variations below the normal are necessary, so that if these be preserved, a less differentiated (*i.e.* retrogressive) type of organism must be produced. The occurrence of variations in regressive development is due, just as in progressive, to the physiological exertions of the organism to restore that correlation of the organs which had been disturbed by a change of environment. Now if the change of environment had been a great and sudden one, and resulted in a less complex environment, the organism, in order to become adapted to the new environment, must produce structural variations which are less complex than the normal in order to bring about a less intimate and complex correlation of the organs than had previously existed. Such variations can obviously be produced only as long as the organs continue to be physiologically active; and necessarily their action must cease before the organs disappear. In other words, it is impossible for variations to lead to the total disappearance of an organ, since the very production of variations is dependent upon the physiological exertions of the organ. Thus we must

postulate for regressive, as has been shown for progressive development, a certain maximum amount of variation, the passing of which would cause death.

TABLE XXI. INDIVIDUAL VARIATION IN THE NUMBER OF THE RECTRICES OF CERTAIN N. A. TETRAONIDÆ.

SPECIES.	NUMBER OF INDIVIDUALS EXAMINED.	NUMBER OF RECTRICES.
Centrocercus urophasianus (Bonap.)	1 ♀	16
	2 ♂ ♂, 3 ♀ ♀, 3 juv ..	18
	3 ♂ ♂, 1 ♀	20
Dendrapagus obscurus (Say)	1 ♂	16
	1 ♀	17
	6 ♂ ♂, 4 ♀ ♀, 2 (sex ?)	18
	2 ♂ ♂, 1 ♀	20
D. obscurus fuliginosus Ridgw.	1 ♂	14
	1 ♂, 1 juv. ♂	16
	3 ♂ ♂, 1 (sex ?)	17
	4 ♂ ♂, 4 ♀ ♀, 4 (sex ?)	18
D. obscurus richardsonii (Sab.)	1 ♀	19
	2 ♂ ♂, 4 (sex ?)	20
	1 (sex ?)	21
	1 ♂	22

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THE EGG OF AMIA AND ITS CLEAVAGE.

C. O. WHITMAN AND A. C. EYCLESHYMER.

PREFACE.

THE search for the eggs of *Amia*, begun by Louis Agassiz, and renewed from time to time by others, was first successful May 1, 1887, when the senior author of this paper found nest and eggs in Pewaukee Lake, Wisconsin. There has been no lack of interest in the search on the part of American naturalists, but a number of reasons conspired to delay the discovery. The nature of the breeding-grounds and the habits of *Amia* were little understood, and those most interested in securing the material lived at long distances from the more promising localities.

The difficulties in the way of the first discovery seem to be little appreciated by those who have recently collected these eggs and made free use of information well known to have originated with one of us, but with no acknowledgment, except to parties who were either wholly innocent of anything but borrowed knowledge of the subject or had acquired their first information under our guidance.

So far as we have been able to learn, no eggs of *Amia* had ever been collected or seen before the date above given. No one knew where to look for them, whether in deep or shallow water, under stumps and logs, or in open places, on sandy or marshy bottom. Whether they were to be found free or adhering to roots or leaves, scattered among grass and reeds or collected in nests or beds, no one could foretell. Fishermen had seen the young in swarms along the shores of many of the Wisconsin lakes, but they knew nothing of the nesting habits and could only guess at the time of spawning.

Amia is very shy and nocturnal in habit. Its nests are not designed to catch the eye. One who had never seen them might pass over dozens and fail to notice them. They are often concealed beneath supernatant grass or reeds, and appear, at first sight, like natural depressions. The eggs agree so closely in color with the ground that the inexperienced observer must get close over the nest to see them, and even then he may fail if the water is the least clouded with mud or rippled by the wind.

The male fish, which alone guards the nest, lies motionless at the approach of a boat, and often allows it to pass over him without stirring. If frightened by a jar in the boat or by the movement of the oars, he leaves the nest, but in doing so generally raises a cloud of mud, and darts off under its cover, stealthily, but with wonderful rapidity. The nest and eggs are often thus effectually concealed from view. If this behavior were something special and peculiar to the care of the nest, as one might suspect on first acquaintance, it would serve to indicate approximately its location; but one soon learns that not every streak of mud in the wake of an *Amia* leads to a nest. Indeed, one may search for hours on such trails and find nothing.

Amia is fond of shallow and quiet bays, where the water first gets warm in spring, and hiding-places are easy to find among the reeds and logs. In such places they spend the day in quiet concealment. When approached in a boat pushed forward with great care to avoid noise and disturbance of the water, they may keep their places, if concealed from view, letting the boat pass by or even over them without moving. Sometimes they

steal away, under cover of plants, so quietly as to escape observation. If suddenly surprised they dash off with such speed that one may get no distinct image of the fish, and see only a streak of muddy water marking its path. A few fish escaping in this way often leave the water of a small bay so cloudy that the search for eggs has to be abandoned until the mud settles.

The difficulties of the search for these eggs were, then, not a few, even in Pewaukee Lake, which is undoubtedly one of the most favorable localities, and thus far the only one where they have been found in abundance. In lakes where the water is constantly cloudy, as in the small lakes south of Chicago, the difficulties are so great that, with all our experience in collecting these eggs, we have not yet succeeded in finding a single nest, after looking for them for two seasons. There must be breeding-grounds somewhere in these lakes, for *Amia* is abundant, both young and old.

After collecting for several seasons at Pewaukee, and after much searching in the rivers and other lakes of Wisconsin and Illinois, we are not greatly surprised that the eggs of *Amia* escaped early discovery. But how did it happen that the discovery once made was not forthwith announced, and followed up with the usual haste to secure priority? Although the fact was not announced in print, no secret was made of it, and it soon became known to many American and European naturalists. As to "priority," no apology need be offered for indifference. "Priority" weighs less and less as investigation weighs more, and, so far as the egg of *Amia* is concerned, there appears to be little reason to envy either the methods or the results of priority-seekers.

To those who have known where these much coveted eggs could be found in abundance, and who have courteously refrained from helping themselves, out of respect for first claims, a word of explanation is due for the failure to bring forth results at an earlier date.

When Mr. Allis started his Lake Laboratory at Milwaukee, under the direction of Mr. Whitman, it was suggested by the latter that the eggs of *Amia* and *Necturus* would be two as interesting subjects for embryological research as could be

found in the lakes of Wisconsin. It was decided, in case the eggs could be found, that Mr. Allis should devote himself to *Amia*, and Mr. Whitman to *Necturus*. Mr. Allis knew the lakes and the fishermen, and was thus most helpful in directing to favorable localities. It was not long before the eggs of both *Amia* and *Necturus* were found. During the first season only a few *Amia* eggs were obtained, but the young were to be had in great numbers. It was decided, therefore, that Mr. Allis should begin his study with the development of the "lateral line" system, for which there was abundant material, and wait until the next season before attempting to trace the development of the egg. Mr. Allis became deeply absorbed in this study, and when the time came for collecting eggs again, the work had progressed to a point where it could not to advantage be laid aside for the tempting study of the eggs. A third season came, and still there was no room for eggs, although a fairly complete series had been collected by Whitman and his assistant, Dr. Patten. At length the work on the lateral line was brought to a close; but just then Mr. Allis's health broke down, and he was advised by his physician to go to Europe for rest and for expert treatment of his eyes. From that time to the present he has found it necessary to prolong his stay, but not without maintaining a private laboratory, and, by the aid of assistants, bringing another anatomical work on *Amia*, of great extent and value, to completion. Beginning with the second year of his absence, Dr. Ayers had charge of the laboratory in Milwaukee, with Eycleshymer, Strong, and Nomura as assistants, and continued the collection of *Amia* eggs. A large number of elegant drawings of the egg were made by Mr. Nomura, and the various stages were systematically sectioned, and several cases of slides were prepared by Eycleshymer and Strong, ready for study the moment Mr. Allis should be able to return. The second plate of the present paper represents some of Mr. Nomura's work, directed by Dr. Ayers and Mr. Allis, and generously placed at our disposal by Mr. Allis.

The material for the study of the embryology of *Amia*, now in the possession of Mr. Allis, covers all stages, and the work already done upon it is considerable. In order to keep this

and related work moving, Mr. Allis kept the Lake Laboratory open for the first four years of his absence, and then closed it, with the expectation of reopening it as soon as he could resume personal direction. All the while he has been pushing forward the anatomical work on *Amia* (now completed and in press), and has thus prepared himself in quite an exceptional way for a thorough study of the development.

Those who know how faithfully Mr. Allis has pursued his work under unexpected difficulties, and how he has continued for years to divide his income in support of the investigations of others, and in maintenance of a national medium of publication, — those who are aware of all this may wonder that a report could get into circulation to the effect that the *Amia* material was being unfairly monopolized. The implication extends to all who have participated in the discovery, collection, and elaboration of this material (Whitman, Patten, Ayers, Eycleshymer, Strong, and Nomura), for all have abetted the crime, in so far as they have refrained from snatching the material themselves or assisting others in such business. The prize of priority, coupled with dishonor and theft, was not a distinction coveted at the Lake Laboratory. If to find what others have failed to find, and to devote life and fortune to its investigation, is unjust monopoly, then how might biology prosper in the exchange of footpads for monopolists?

Those who, knowing all the circumstances, have nevertheless given currency to this censure, or allowed it to pass undisputed, have something less than generous instincts to be proud of. Mr. Allis's great crime reduces itself to the misfortune of having had to submit to delay in his work, to the neglect of all tinnabulous advertising, and to contempt for the notoriety accorded to prolific scribbling and priority hustle. To all courteous applications for *Amia* material Mr. Allis has freely responded to the extent that his resources and work would permit.

It remains to deal briefly with Dr. Fülleborn's mission for the collection of the eggs of *Amia* and *Necturus*. From what has already been stated, it will be clear that there were certain claims and obligations in relation to this material which were not to be entirely overlooked. Without a word of previous

notice or consultation with the parties primarily interested, Dr. Fülleborn came to us with the request for direction and aid in getting the eggs. Under the circumstances, we could only decline, explaining the reasons which stood in the way, and pointing out some of the proprieties in the case which should have received attention. It is quite possible that Dr. Fülleborn had not been previously informed of these, as was to be expected that he had been by Dr. Virchow, under whose instruction he was acting.

When, in 1893, Dr. Virchow applied to several parties for assistance in finding the eggs of *Necturus* and *Amia*, he was referred to Mr. Whitman. Learning that work on these eggs was already in progress, Dr. Virchow courteously explained that he only wished to study the "Dotterorgan," and that he would not make any use of the material which would conflict with the work on general development already begun by Mr. Whitman. With that understanding, the information desired was freely and fully given, and a pretty complete series of drawings of the egg of *Necturus* was shown to him.

Next came Dr. Fülleborn, under advice from Dr. Virchow, to take advantage of the information confidentially obtained by the latter, in collecting the same material for purposes well known to conflict with studies in progress here. The mystery of the "Dotterorgan" was now disclosed, and no further comments seem to be required.

OECOLOGICAL OBSERVATIONS.

1. *Previous Papers.*

There are only four works to which we can refer the reader for information on the habits, eggs, nests, and larval history of *Amia*. Mr. Allis¹ has given a series of drawings of the larva, to illustrate the successive changes in form and the development of the lateral-line organs. Dr. Fülleborn² has recently

¹ Edward Phelps Allis, Jr.: The Anatomy and Development of the Lateral Line System in *Amia Calva*. *Journ. of Morph.*, II, p. 463. 1888.

² F. Fülleborn: Bericht über eine zur Untersuchung der Entwicklung von *Amia*, *Lepidosteus* und *Necturus* unternommene Reise nach Nord-America. *Sitzungsberichte der Akad. d. Wiss. zu Berlin*, XL, pp. 1057-1070. Oct. 25, 1894.

reported his experience in obtaining the eggs, together with observations on the habits, times, and places of breeding. This author describes the floating islands in Pewaukee Lake and Fowler Lake and their labyrinth of canals, and speaks as if *Amia* was so select in its choice of breeding-ground that its nests were to be found only in these canals. That is not the case, however, in either of the lakes just mentioned, and it is quite certain that *Amia* breeds in many places where there are no floating islands with canals, — for example, La Belle Lake, which Dr. Fülleborn cites as not containing a single nest.

The spawning season is said to have extended from the beginning of May to the first days of June in the summer of 1894. According to our observations, the best time for collecting generally falls between the middle of April and the end of the first week in May. We have collected eggs the last week in March and the first week in June, but these extremes mark unusually early or late seasons.

The time for hatching is estimated to vary between six and fourteen days. This is a variation wide enough to include the truth without hitting it in either direction. Surely one season's experience ought to have reduced both margins of this conjecture, and furnished an answer to the question how long a time is ordinarily required to hatch these eggs.

The nests described as "not yet filled with eggs," in which male *Amiae* were found, were probably nests in which the young had hatched. The observation furnishes no ground for the belief that the male alone makes the nest. The statement that the nest is always open to the sunlight does not accord with our experience, and the opinion that young and old seek deep water after the first of June is entirely erroneous. The assertion that nests containing "nur verschimmelte Eier" were still guarded by males is another error due to superficial examination. Such eggs are frequently found in nests containing newly hatched larvae, and deserted nests are occasionally occupied as convenient resting-places during the day. It does not follow because an *Amia* is found in a nest that it must be there for the purpose of guarding it.

The statement that the male takes its brood to the shore in order *to sun itself and them* would adorn a nursery tale better than the Proceedings of an Academy of Sciences. Dr. Fülleborn does not appear to have discovered that *Amia* prefers the shadows of evening and the darkness of night to the glare of the sun. *Amia* places its nest near the shore for warmth, not for light; it leads its young along the shore for food,* not for basking in sunlight. It is towards evening and in the early morning that the swarms of young may be seen to best advantage. As they grow older and more wary they may shift their feeding-ground, not to deep water, but to new places along the shore. Their seeming to disappear is accounted for by the fact that the individuals of a brood wander more widely apart as they grow older and require more food, and at the same time they become increasingly shy, and hide themselves beneath the banks, or whatever lies nearest, long before a boat in motion can be brought within seeing distance. The boat must come to rest and the observer must sit motionless by the half-hour if he desires to see young *Amiae* feeding in June. A slight jar of the water is enough to send hundreds of these young fish out of sight in a flash.

The mistake in supposing that young *Amiae* retreat to deep water soon after June 1 led Dr. Fülleborn quite astray on another point of considerable importance. The conclusion that the young go straight on to become more and more like the adult in both form and color is about as wide of the mark as it could be. The changes in color exhibited at different ages during the summer and autumn are as characteristic and remarkable as the changes in plumage among birds.

Dr. Fülleborn is careful to note that the young larvae with large yolk-sacs, before leaving the nest, have a distant resemblance to tadpoles, but he neglects to mention that this resemblance becomes even more striking after the yolk-sac has disappeared and after the nest has been abandoned. The larvae, shortly after leaving the nest, while moving about in a dense swarm, resemble tadpoles so closely in form, color, and

* The food of the young consists mainly of daphnia and cypris, as Dr. Ayers informs us.

motion as to be easily mistaken for them when seen at a distance of some yards, as from a boat. A stranger to the fish and its habits might wonder at the closeness of the swarm, but, if he did not stop to examine, he would take them for tadpoles.

Dr. Fülleborn was in such haste for priority that he could not stop to settle the important question as to whether the first cleavage grooves actually reach the vegetative pole of the egg. "The first grooves," he says, "appear indeed to reach the vegetative pole; but the investigation has not advanced far enough to admit of definite statements." This question might have easily been settled on the living egg, and if the material collected was so poorly preserved as not to admit of positive statements on this point, it may be more "complete" than instructive.

Only a little over four pages of this preliminary paper were devoted to *Amia*,—certainly enough to show the need of more careful observation.

The third paper to be mentioned in this connection is that of Bashford Dean,⁸ who affirms several times that he has fully confirmed the statements of Fülleborn on the "general" and "spawning habits of *Amia*." Perhaps the above remarks are not superfluous after such confirmation. As Dr. Dean seems to have depended a good deal upon what "the fishermen stated," the confirmations offered are not always very confirming.

It should be said, however, that the fishermen of Pewaukee and Oconomowoc have had many opportunities for instruction since 1887, and some of them are now better informed on the habits of *Amia* than they were found to be at that time. Some of the statements credited to them are entirely accurate, some are crude guesses, and others pure "fish stories." The following will serve as examples:

"The fish were observed depositing their eggs as early as April 25, and before the 1st of May the spawning appeared to have been generally completed." Although no authority is quoted, this is evidently a statement based upon a fisherman's story. It so happened that we collected eggs and larvae from

⁸ Bashford Dean: The Early Development of *Amia*. *Quart. Journ. Micr. Sci.*, XXXVIII. February, 1896.

the same locality during the week preceding Dr. Dean's visit in the spring of 1895. On the 12th day of May, between 4.00 and 5.30 A.M., a number of broods of larvae were caught, the largest of which measured 25 mm. These must have been at least 35 days old, as a glance at our table, p. 327, will show. These facts show beyond question that eggs were laid during the first days of April.

"At the height of a 'run' as many as a half-dozen nests, as *fishermen stated*, were found to occur within the space of a few square yards." This needs confirmation. We have never found the nests in such close proximity, — never more than four or five in a single bay, and usually rods apart. The small bays along the shore of Pewaukee Lake generally furnish ground for but one, or at most two or three nests.

"Immediately before spawning, *it is said* that the fish divide themselves into parties, each comprising a female and several males, and that they then circle about nearer and nearer the shallows." True to the extent that the female may be contended for by two or more males. They are on the "shallows" to begin with, and hence do not need to "circle about" to get nearer to them.

To say that "the fish divide themselves into parties" comports well with a fisherman's yarn, and shows how useful such yarn may be in darning up gaps in observation.

"The mode of building a nest is in some ways doubtful; *fishermen state* that the spawning party prepares it by constant circlings before the time of spawning, and this view seems entirely corroborated by a careful examination of the newly made nest; the soft weeds and rootlets appear bent and brushed aside in a way that gives it somewhat the appearance of a crudely finished bird's nest."

That the nest is built, and built with considerable care in many cases, is evident enough. "The mode of building," however, is, indeed, doubtful; no less doubtful at the end than at the beginning of the fisherman's tale.

"And it seems evident that nests are prepared sometimes well in advance of spawning, for several were noted by the writer which were occupied by the fish for a number of days

before the eggs were deposited." Dr. Dean does not give us any information as to the number or sex of the fish which he observed in these nests. From his statement, page 414, viz.: "By the time of my visit, however, the spawning season had practically ended," we are led to suggest that these nests might have contained newly hatched larvae. We have never observed either the male or female occupying a nest for a number of days, or even one day, preceding deposition.

"The mode of depositing the eggs appears to be entirely similar to that described by the present writer in the case of the gar-pike. The spawning fish leaves the nest from time to time, returning in company. The eggs and milt are emitted simultaneously. The fishes apparently rub close together, since scales are found scattered in the nest bottom, as noted by Fülleborn, and as now confirmed by the present writer." How much of this is to be understood as personal observation the author seems to avoid making clear. The words "appears" and "apparently" look more like conjecture than anything else. Leaving the nest from time to time could hardly apply to the gar-pike, which has no nest. "From time to time" suggests that oviposition occupies considerable time; and the author conjectures that in some cases as many as twelve hours are thus consumed. In this he is probably much mistaken, as will appear later on. The occurrence of scales in the nest has an entirely different explanation from the one above suggested, as an observation made by Dr. Eycleshymer, to be related presently, will make evident.

The nest represented on page 419 is so great an improvement on nature's art that one might readily conclude that it was formed by "constant circlings." The essential thing in an *Amia* nest is fine rootlets or grass for holding the eggs free from mud. If the fish finds a surface adapted to its needs it may use it without making any excavation whatever. Such nests are often found along the submerged edges of the floating islands, or "bogs," as they are locally called. Along the shore of the lake the nests are sometimes placed beneath a log or stump, but most frequently by the side of a clump of reeds. Sometimes the fish finds rootlets only around the edges or on

one side of its nest, in which case no eggs will be found on the bottom. When excavations are made the depth may vary from a few inches to a foot, depending upon how much mud and weeds have to be removed in order to get the surface required for the eggs. The nest is generally from 50 to 60 cm. in diameter, and more or less circular, varying considerably in adaptation to the conditions of the ground.

The author's remarks on the breeding habits end with a bit of romance funny enough to claim a place beside the story of Dr. Estes, which will be given presently.

Dr. Dean says: "A fine nest of eggs was found to be entirely deserted at a time when the young could not have been older than twenty-four hours. The closest search in and about the nest revealed no trace of their whereabouts, although from their larval habits it was thought that they should surely be found attached to the neighboring weeds, or deep in the mass of root fibres and detritus of the nest bottom. They had evidently left the nest in a body, and were nowhere in the immediate neighborhood. It was plausibly suggested by Mr. G. W. Kosmak, who then accompanied the writer, that they had been taken away by the male fish, attached to him by their sucking-disks. It is certain that when the male reappears it is with a swarm of nestlings; but they are now well grown ($\frac{3}{8}$ to $1\frac{1}{4}$ inches)."

The earliest paper dealing with the habits of *Amia* to which we have to refer is one cited by Dr. Goode from the Sportsman's Gazetteer, pp. 324-326, 1887.⁴ It is here that the story of Dr. Estes, above alluded to, is found.

"The best description of the habits of this fish," says Goode, "is here quoted from the pen of Charles Hallock:

"They take frogs, minnows, and sometimes the spoon. Their habitat is deep water, where they drive everything before them. They are very voracious and savage. Their teeth are so sharp and their jaws so strong that they have been known to bite a two-pound fish clean in two the very first snap. They are as tenacious of life as the eel. The

⁴ Geo. Brown Goode: Natural History of Useful Aquatic Animals, pp. 659, 660. 1884.

young, when about six inches long, make a famous bait for pickerel and pike. To use it, run the hook into the mouth right up through the centre of the head, through the brain, cast a hundred times, catch several fish, and at the end of three to six hours he will kick like a mule. Put one hundred in a rain-barrel and you can keep them all summer without change of water. For the aquarium the young have no equal, and on account of the spot in the tail are quite attractive; but nothing else but snails can live in the tank. He will kill a lizard or any other living thing the instant it touches the water.'

"Dr. Estes says: 'I have sent these young dogfish hundreds of miles for the aquarium. It is only necessary to keep them in water, a change scarcely being required. The adults are the great "jumpers" of the lake. On certain days they are to be seen in all directions jumping clean out of the water, and turning complete somersaults before again striking. *They spawn in May and June among the grass and weeds of the sloughs, if they can reach them in time. As soon as the spring rise comes, usually in May and June, and connects the inland sloughs with the lake (Pepin), they run up and over into the sloughs, deposit their eggs, and remain near the beds and young just as long as they can and not be shut in by the receding water. The eggs hatch in eight and ten days, the parents remaining with the brood two or three weeks, if possible, but will leave them much sooner, if necessary, to save themselves. The young will not make any effort to escape to the lake until the next season, when, if an opening occurs, they come pouring out in countless numbers. At this time we take them by stretching the minnow seine across the opening and raising it when full. They are now from three to six inches long, fat and chubby. I come now to mention a peculiar habit of this fish, no account of which I have ever seen. It is this: While the parent still remains with the young, if the family become suddenly alarmed, the capacious mouth of the old fish will open, and in rushes the entire host of little ones; the ugly maw is at once closed, and off she rushes to a place of security, when again the little captives are set at liberty. If others are conver-*

sant with the above facts I shall be very glad; if not, shall feel chagrined for not making them known long ago.' "

The author of this amusing story is evidently quite blind to the part played by his fancy. The fish has no such strange habit, and the wonder is that any sane mind could have invented such a prodigious fabrication without realizing that the whole thing was a myth. The facts from which the story was concocted are: (1) The fish sometimes opens its mouth on approach (as if threatening an attack); (2) the young suddenly disappear (in the mud and weeds of the bottom); (3) the old fish darts off and is quickly lost sight of; (4) the old and young are found a little later at a short distance from the place where they were first seen.

The young fish disappear so quickly that the observer fails to see where they go; the reappearance of the brood in company with the parent is taken as proof that he took them with him, and the open mouth as proof that they were swallowed.

2. *Behavior during Spawning.*

Although the early cleavage stages of *Amia* have been repeatedly collected, it was not until May 12, 1895, that the behavior of the fishes immediately preceding and during spawning was witnessed. The observation leaves certain points in doubt, but fills some of the gaps in the records hitherto made.

Starting at daybreak on the morning of May 12th, we soon arrived at the "bogs." After taking a number of broods of larvae, we were slowly passing through a narrow channel which in its deepest portion did not exceed 60 cm. This channel was filled with dead grass from the preceding year, while the growth of the present season was barely visible above the surface of the water. On one bank the green grass grew in profusion, on the other the dead rushes formed a thicket. While resting for a few moments a disturbance of the water not more than 3 m. distant attracted our attention; stepping to the bow of the boat we saw four large *Amiae* lying in a nest. Seeing us they left the nest and disappeared in the surrounding grass. Soon three of them returned, but after a few brisk movements

again retreated, this time making short circuits in the adjoining grass and frequently thrusting their snouts above the water. We now easily ascertained that the party consisted of two males and one female.* After a short interval the three again returned, when a fierce battle for supremacy ensued between the males. They approached from opposite sides of the nest and locked jaws in a most ferocious manner. Their struggles were so violent that a cloud of muddy water soon arose and obscured them from view. When the female, which during the battle had remained concealed at the side of the nest, again came into view, the victorious suitor rushed at her and began to bite her sides with so much vigor that a number of scales were detached. The two then swam slowly about the nest, keeping their bodies in close proximity. At short intervals this movement was interrupted by momentary periods of vigorous activity. This lasted some five or six minutes, when they departed. Believing this to be due to unavoidable movements of the boat, we thought it best to retire and leave them undisturbed.

Returning after twenty minutes, and moving with greatest care, we approached and found the male lying at full length in the nest. Occasionally he would move slowly around the area, keeping up a very rapid movement of the pectoral and pelvic fins. We expected that our approach would frighten him; but not until the boat was directly over him did he show any signs of vacating. It was now plainly to be seen that the nest was filled with freshly deposited eggs. It is probable, though not certain, that no eggs had been deposited at the time when we first saw the fishes in the nest. The oviposition most probably took place after the battle of the males, and during the time (5-10 min.) the female was in the nest with the victorious male. That the average period of deposition is brief can hardly be doubted, since in most cases the eggs of a nest are found in the same or nearly the same stage of development. In one nest, however, we found some of the eggs in a late stage of cleavage, while in others the embryo was already visible.

* The male is easily distinguished by a dark spot in the upper portion of the caudal fin. This spot is fringed by a ring of dull orange, which during the breeding-season assumes a brilliant hue.

We inferred that two females sometimes deposit in the same nest. This view is corroborated by the fact that in this case the number of eggs was about double the average number deposited by a single fish.

The eggs were scattered over an area about 30 cm. in diameter, the greater portion being deposited upon the grass lining the sides of the nest.*

Dr. Ayers has kindly given us the following observations on the breeding habits of *Amia*, which supplement the foregoing statements:

"The nest of *Amia* is built after pairing, and during the preliminary steps to egg-laying. The nest is not a premeditated structure, but merely the result of the movements of the fish in and about the place selected for spawning during the period of sexual excitement, which ends in the emission of eggs and sperm. How long the movements continue I am unable to say positively, but from many observations I conclude that they usually last from two to three hours. The nest-forming process generally begins at early dawn, and the eggs are cast about sunrise. I have observed some cases, however, in which the eggs were deposited in the afternoon.

"*Amia* usually selects grassy fields on which to deposit its eggs. After the eggs are cast, the male fish usually remains in the neighborhood of the spawning place, swimming in a circle, and frequently uttering a series of short, snorting sounds, which are probably intended to attract the female. The female, however, does not usually return to the spawning place, but swims away, and the male fish is left to guard the eggs. The male fish usually remains in the neighborhood of the spawning place for several days after the eggs are cast, and during this time it usually utters the snorting sounds mentioned above.

"In Mississippi the eggs are usually deposited in the mud.

*The eggs were deposited in the grass, and not in the mud, as is usually the case.

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which the pair were still engaged in spawning. Eggs were taken from the nest and at once placed under observation, and the following record made: Three sets of eggs were put in glass dishes, and Mr. Nomura, Mr. Eycleshymer, and myself observed independently the segmentation of these fresh-laid eggs. The first segmentation groove began at 5.30 P.M. The second meridional began at 6.30 in those eggs which were placed in the sun after the beginning of the first furrow, and at 7 o'clock in those eggs kept in the shade out of the sun's rays.

"The third and fourth meridional furrows began simultaneously at 7.30. The first equatorial to be seen made its appearance at 8.25, and completed the circuit at 8.40. The next set of eight peripheral meridional furrows began at 9 o'clock, and the second apparent equatorial furrow began at 9.30.

"Amiae are to be found in Oconomowoc Lake during the winter months, living in schools closely huddled together in the bottom of pockets or shallow depressions of the gravelly bed of the lake, among the water weeds. These fish may be speared from a boat, or, if the lake is frozen over, through holes in the ice. They lie so close together that occasionally two individuals are impaled on the fish spear by one throw. When thus disturbed they scatter from their resting-place, moving out a short distance, to return quickly after the first few disturbances. When alarmed by repeated thrusts of the spear, they remain away for hours at a time. Why they should be so tenacious of these resting-places will probably be explained when we know why they choose them in the first place. It is possible that the temperature of the water is higher in these places than elsewhere, owing to the entrance of spring streams into the lake bottom."

3. *Some Further Data.*

In some of the eggs taken from this nest, and kept under close observation, the first indication of the first cleavage appeared at the upper pole at 7.54, *i.e.*, 2 hrs. 24 min. from the supposed time of deposition. At this time the first cleavage

had already begun in many of the eggs, while in others it had not yet appeared.

At the end of 10-15 hrs., 100-150 cells have been formed in the upper hemisphere, while in the lower, 15-20 grooves are present, of which 8-12 have reached the pole.

To distinguish a stage which marks the beginning of gastrulation is somewhat difficult; if the stage shown in Cut 12 might be considered as such, the time would be 35-40 hrs. after deposition. The process covers a period of 30-40 hrs.

In 70-80 hrs. after deposition the embryo appears. On the 7th or 8th day its first movements are visible; these occur at long intervals, and last only a few moments. The periods of activity become more frequent and vigorous until the 8th or 9th day, when the envelope is ruptured and the larva escapes. The larva at this time measures 5-6 mm. Its body is pale flesh color (without pigment), while the large yolk sac is deep sepia. The larvae, as soon as they are hatched, attach themselves, by means of their adhesive discs, to the first object met, and adhere so firmly that it requires some effort to shake them loose. It is impossible at this time for the larva to move its load of food yolk. Once losing its hold, it falls heavily to the next object, where it again attaches itself.

At the time of hatching one frequently sees the male fish carefully guarding his nest, in which there apparently is not a single living egg or larva, the only evidence of their former presence being the fungus-covered debris scattered here and there. A careful search, however, among the rootlets will enable one to discover the newly hatched larvae.

During the 10th or 11th day the larva reaches a length of 6-8 mm.; pigment begins to appear in the retina and other parts of the head. The pectoral fins have shifted from the horizontal to an oblique position; the movements are less vigorous but more frequent.

In 12-15 days a length of 9-11 mm. is reached. They are now deeply pigmented with dark brown. The pectoral fins have taken a vertical position. The yolk sac has changed from the spherical to an ovoid form, with its smaller end posterior,

and is soon absorbed. About this time the larvae are ready to leave the nest with the male fish. Frequently we have seen the entire brood, generally numbering several hundred, huddled so closely together that they formed a black mass moving along in close company with the parent. If disturbed, the parent rushes off, setting the water in commotion by its movements, and the brood scatter to conceal themselves at the bottom. In a short time, if one remains perfectly quiet, he may see the larvae come forth in small groups from their hiding-places, and perhaps the return of the parent.

The following table shows the rate of development. The cleavage times are based upon observations made on a single egg, while the times recorded beyond late cleavage are based upon data obtained by following the development of a second egg.

AGE IN HOURS.	LENGTH IN MM.	STAGE OF DEVELOPMENT.
—	—	Oviposition, beginning of
2.24	—	First cleavage, " "
3.28	—	Second " " "
4.20	—	Third " " "
5.22	—	Fourth " " "
6.20	—	Fifth " " "
7.25	—	Sixth " " "
10.	—	Late " "
15.	—	Blastula
40.	—	Gastrula, early
70.	—	Embryo visible
160.	—	Embryo begins to move
200.	5-6	Embryo hatches
250.	6-8	Appearance of pigment
360.	9-10	Volk-sac absorbed
480.	12-14	Pelvic fins appear
720.	17-20	—

CLEAVAGE.

Our observations on the cleavage comprise : first, a study of surface phenomena, followed continuously on the living egg ; secondly, a comparison of corresponding stages as traced on prepared material ; and thirdly, a study of serial sections.

In following the cleavage of the living egg we found it convenient to proceed as follows : The eggs still attached to blades

EXPLANATION OF CUTS 1 TO 6.*

CUT 1. — Vertical section of the mature ovarian egg, showing the calotte, the germinal vesicle, and a portion of the yolk.

CUT 2. — Vertical section of an egg at the beginning of the second cleavage. The position of the section is shown by the dotted line in *Diagr. A*. It shows the thickness of the calotte, the depth of the first cleavage groove (I) and the vacuolar spaces.

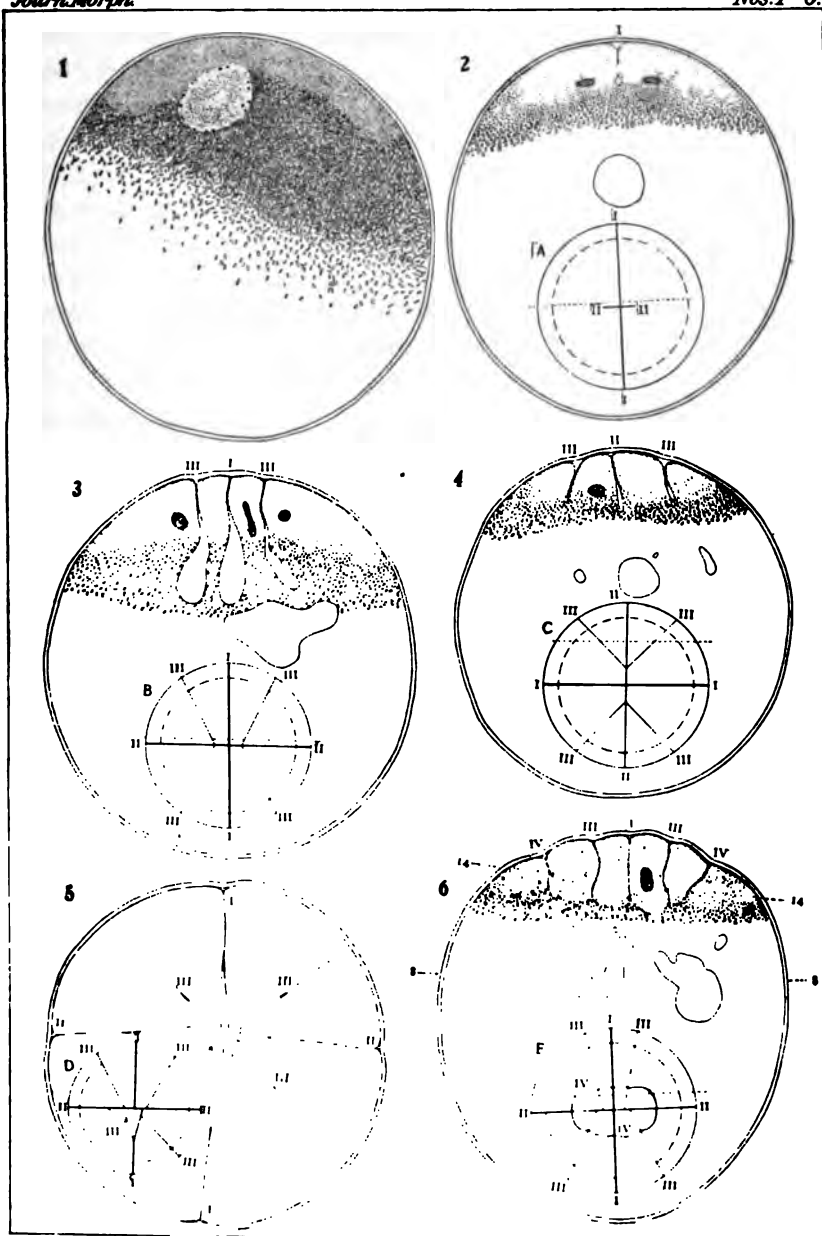
CUT 3. — Vertical section in the stage of the third cleavage, in the plane of the dotted line in *Diagr. B*. It shows the depth to which the first (I) and third (III) cleavage grooves have extended, having now cut through the calotte and become continuous with the vacuolar spaces, which have enlarged and increased in number.

CUT 4. — Vertical section of an egg in the same stage, but with the third verticals (III) arranged as in *Diagr. C*. The section passes near the margin of the calotte (dotted line, *Diagr. C*). The cleavage grooves have barely cut through the calotte, and the vacuolar spaces are less numerous than at the centre of the egg.

CUT 5. — Horizontal section of an egg in nearly the same stage. In this case one of the third set of grooves (III), instead of taking a radial direction like the others, occupies the position shown in *Diagr. D*. The section passes just below the calotte, and shows that the cell at this point is not yet separated from the yolk.

CUT 6. — Vertical section of an egg in the stage of the fourth cleavage, in the plane of the dotted line in *Diagr. E*. It will be observed that the cleavage groove passes somewhat obliquely through the cell, cutting two cells at the margin. An examination of another section of the same egg at this stage shows a lower cell, a contrast to the one at the margin, which is not yet separated from the yolk.

* The position of the section is shown by the dotted line.



of grass or rootlets are placed in shallow watch-glasses and held in a fixed position by weighting with small pebbles. The watch-glasses are then placed upon a mirror fastened to the stage of a dissecting microscope. We could thus observe the changes occurring on opposite sides of the egg without disturbing its position.

The fixing fluids which have given the more satisfactory results are: Flemming's fluid, Perenyi's fluid, chrom-osmic, picro-acetic, and picro-sulphuric. For surface views chrom-osmic fixation gives most perfect pictures, the osmic blackening the cleavage grooves so that they stand out in bold relief. Another excellent method is surface staining with Delafield's haematoxylin, which may be employed with any of the above-named fixatives. For material which is to be sectioned, picro-acetic and Perenyi have given best results. Owing to the crumbling of the yolk we have been obliged to use celloidin as an imbedding mass. Serial sections by the method described by the junior author⁶ have been used for the most part. Staining in section with Ehrlich's haematoxylin and Mayer's alcoholic carmine has proved most satisfactory.

The freshly deposited egg (Fig. 2) is firmly fixed to the object with which it first comes in contact by means of the threads of the villous layer, which are elongated over the lower hemisphere of the egg membrane. The egg is oval in profile view, measuring in its longest diameter, including the membrane, 2.5 to 3 mm.; in its shortest, 2 to 2.5 mm. The upper pole of the egg, "calotte" of Fülleborn, "germ-disc" of Dean, is light yellowish brown. This calotte shades off at its margin into the dark grayish brown of the yolk. Near the centre of the calotte there is a single micropylar orifice, as shown in Figs. 21 and 22, which remains visible for some time (Figs. 12 and 16). We have frequently noticed that the margin of the calotte extends farther toward the equatorial region on one side than on the other, as shown in Fig. 2.

The calotte is already present in the full-grown ovarian egg shown in Cut 1. It is not of quite uniform thickness, but is a

⁶ A. C. Eycleshymer: Notes on Celloidin Technique. *American Naturalist*, vol. XXVI, pp. 354-358.

little thicker on one side than elsewhere. The germinal vesicle lies eccentrically beneath the deeper side, mainly in the yolk, only its upper surface projecting into the calotte. There are thus indications of a definite orientation in the egg before, as well as immediately after, fertilization.

First Cleavage. — In the egg shown in Fig. 3 the first cleavage appeared at 7.54 A.M. (2 hrs. 24 min. after deposition). A slight flattening of the animal pole precedes the appearance of this groove, which generally divides the calotte into nearly equal portions. The groove travels more and more slowly as it passes beyond the margin of the calotte. Its decreasing rate of progress in the egg figured is indicated by the following percentages, based upon the length of the arc traversed at successive intervals of about 1 hr. each, as compared with the entire circumference. At 8.53 (Fig. 3) the arc described equals 36% of the entire circumference; at 9.49 (Fig. 4) 60%; at 10.50 (Fig. 5) 77%; at 11.55 (Fig. 6) 89%; between 11.55 and 12.58 (Fig. 7) 100%. In most cases this cleavage is not complete until after the appearance of the fourth and sometimes even the fifth set of grooves.

The first groove is most frequently meridional, as shown in Figs. 3, 21, 22, and 23. In some cases, however, it may depart so far from a meridional that the segments formed are quite unequal (*cf.* Dean, *l.c.*, p. 425), as shown in Fig. 9. The ends of the groove may meet at the lower pole so as to form a continuous straight line, or in such a manner that an obtuse angle is formed (Figs. 14, 17). Sometimes they do not meet at all, and are only united by a portion of the second, or even third, cleavage grooves.

At the time the second groove appears the first has a depth of about one-half the thickness of the calotte, as shown in Cut 2 (made in plane of dotted line of Diagr. A). The path of this groove is definitely premarked to a point about twice this depth, terminating in an irregular vacuolar space at the level of the nuclei. The depth of the cleavage at this time strongly reminds one of the conditions seen in teleostean eggs with a perfectly defined blastodisc. The calotte certainly represents a concentration of germinal material, analogous to the blasto-

disc seen in the meroblastic egg of the teleost. One might say that the calotte represents a nascent blastodisc, — a blastodisc *in statu nascendi*, so to speak. It differs from the blastodisc, as seen in pelagic teleostean eggs, chiefly in not containing the whole, or almost the whole, of the material to be used in the formation of the embryo. The *Amia* egg is holoblastic, but represents an advance upon the condition seen in the egg of *Acipenser*, in the direction of the meroblastic egg of the typical teleost.

At this stage one or more irregular cavities are found in the upper hemisphere, between the centre of the egg and the calotte. One of these is shown in Cut 2. Just when and how these cavities arise we cannot say, but they are present in the first, as well as the later stages of cleavage. Sooner or later they become continuous with cleavage grooves, and in many cases unite in a common cavity. As these cavities appear in eggs prepared in different ways, collected in different seasons from various nests, it seems certain that they are not to be considered as artificial.

Second Cleavage. — The two grooves of the second cleavage usually begin at the same point of the upper pole and extend meridionally at right angles to the first groove (Figs. 4, 22, 23). Their progress is essentially the same as that of the first. In the egg shown in Fig. 4 these grooves appeared at 8.58; at 9.40 they had extended slightly beyond the margin of the calotte, and three to four hours later they had completely encircled the egg.

Variations in the formation of these grooves are not uncommon. The point of origin may be at a greater or less distance from the pole (Fig. 10). Instead of a right angle, acute and obtuse angles may arise, the grooves crossing in the form of an X (Fig. 11). Again, the furrows may not arise at the same point, but at points more or less widely separated, as in Fig. 12. They do not always begin at the same time; one may precede the other by a considerable interval, reaching near the lower pole before the other has passed the equator (Fig. 14). Occasionally the two unite at the lower pole to form a continuous line running at right angles to the first; at other times they

form acute or obtuse angles, as at the upper pole, or they may even terminate at points widely separated, as in Fig. 17. In some cases the grooves may not even reach the lower pole, but, diverging from a meridional plane, unite with the first groove at almost any point between the margin of the calotte and the lower pole.

Third Cleavage.—By the time the second grooves have passed just beyond the margin of the calotte the third set of grooves appear. In a majority of cases these are vertical, and occupy the positions shown in Figs. 5, 13, 15, 23, 24, and 25. They generally all depart from one or the other of the first two meridionals, thus giving rise to a distinctly bilateral appearance. In the egg followed (Fig. 5) the first of this set originated in the first meridional at 9.50. The second was formed in the adjacent quadrant, on the opposite side of the first meridional, at 9.51. The third appeared one minute later in the adjacent quadrant on the same side of the first meridional. The division of the remaining quadrant began at 9.54. The two furrows shown in Figs. 5, 6, and 7 later reached the lower pole, but the one first formed turned aside, running into the first meridional at some distance from the lower pole, while the remaining one united with the second meridional at about the level of the equator.

It often occurs that one or more of the set depart from the first meridional, while the rest depart from the second, or *vice versa* (Figs. 13 and 24). Frequently one observes the condition shown in Fig. 15, where all the grooves of this set pass in planes nearly parallel to the first or second meridional, giving rise to a bilateral form comparable with the 8-cell stage of the teleostean egg.* All these grooves may occasionally depart from a common point at the upper pole, and cut each quadrant of the calotte into two approximately equal portions, in which case the cleavage assumes a radial form such as has been described by Agassiz and Whitman[†] in the pelagic fish egg. Rarely, if ever, do these grooves divide the quadrants equally at the lower pole.

* Dean (No. 3, p. 425) speaks of this condition as if it were the general rule.

† Agassiz, A., and Whitman, C. O.: On the Development of Some Pelagic Fish Eggs. *Proc. Amer. Acad. Arts and Sci.*, XX, pp. 23-75. 1884.

Cut 3 represents a vertical section of an egg at a stage just preceding the fourth cleavage. Its position is shown by the dotted line in *Diagr. B*. The section runs parallel with the second meridional, and cuts through two of the cells delimited by the third verticals (*III*). The first meridional has at this time cut entirely through the calotte and deeply into the underlying yolk. The third set of grooves have likewise extended far into the yolk. These grooves usually pass in vertical planes; at times, however, they are more or less inclined; we have sections in which one or more of them pass so obliquely that the cells are deeply cut, but in no case have we found any of them entirely severed from the underlying yolk. Occasionally one finds eggs in which one of these grooves (*III*^a) occupies the position shown in *Diagr. D*, Cut 5. From surface study one might be in doubt whether this groove is vertical or horizontal. Serial sections of three such eggs show that the groove is vertical, and that the segment in each case is continuous with the underlying yolk.

In Cut 5 a horizontal section of such an egg is shown which passes just beneath the calotte. All the third cleavage grooves (*III*) except one (*III*^a) are arranged radially, alternating with the two primary grooves. It is noteworthy that all these grooves widen into fissures which have a common vacuolar centre, giving rise to a star-shaped figure.

Cut 4 exhibits a vertical section of the same stage, but at right angles to the plane of Cut 3. The grooves on the surface of the egg are shown in *Diagr. C*, and the position of the section is indicated by the dotted line. The section is taken near the margin of the calotte. Sections nearer the centre of the egg show that the first and second grooves have reached a much greater depth, and have become continuous with cavities similar to those shown in Cut 3.

Dean (No. 3, p. 426) states that "the segmentation cavity takes its definite origin at this stage; in the region of the animal pole the blastomeres are separated from the underlying yolk—the germ disc by a narrow fissure, which has been found to arise in the cleavage planes of the animal pole."

Passing over the singular construction of the second statement, which we confess to being utterly unable to comprehend, we are led to make a remark on the "segmentation cavity." It seems evident that Dr. Dean is predisposed to interpret his sections of the *Amia* egg in correspondence with what he finds laid down by Dr. H. V. Wilson in regard to the egg of *Serranus*. The discovery of a "segmentation cavity" in his Fig. 23 seems to have been thus inspired (compare this figure with Wilson's Fig. 15).

If the section shown in Dean's Fig. 23 passes in the plane indicated in his Fig. 4, the cell *a* is cut very near its central margin, and a little obliquity, either in the plane of the section or in the plane of the bounding groove, might be sufficient to account for the appearance. In other words, the section may represent only a chip from the central margin of cell *a*.

We must differ with Dr. Dean in respect to what seems to be his notion of "the segmentation cavity." The fissure, or fissures, thus designated by Dean, as a glance at his Figs. 27-30 will show, represent nothing more than ordinary intercellular spaces. Such spaces, as all the world knows, are not peculiar to any egg, and are not to be confounded with "*the* segmentation cavity." Dr. Dean says in one place (p. 424) "the segmentation cavity is practically wanting." Nevertheless he speaks later of its "definite origin," and calls special attention to it in his figures. In one case (p. 441) it is called a "flattened segmentation cavity."

Dr. Dean represents the segmentation cavity as beginning with the second cleavage but as taking "definite origin" at the time of the third cleavage. In Dean's Fig. 24, representing a section of an egg in the stage of the fourth cleavage, the "segmentation cavity" is shown as intercellular spaces, which he admits "might perhaps be regarded as artifacts." We would suggest that these spaces have a different meaning. The elongated nucleus shown in one of these cells is very clear evidence that what we have called the horizontal cleavage is in progress. At the time of the cleavage a constriction takes place at about the level of these spaces, and it is to these constrictions that they probably owe their origin. There is another

important point to be noticed in connection with this figure. The central blastomeres are represented as cut off from the yolk below. We feel confident that if the entire series be examined the cells will be found to be still continuous with the underlying yolk.

The cleavage cavity in *Amia* has rather an interesting mode of origin. For the first appearance of this cavity is in the form of central vacuoles, which are present as early as the first cleavage groove. The cleavage grooves, in many cases at least, expand into broad fissure-like cavities as they approach the centre of the egg (Cuts 3, 5, 15), where they become continuous with the earlier cavity or cavities. As the cleavage runs on these spaces enlarge, flow together, and thus often give rise to quite a spacious cleavage cavity, such as shown in Cuts 10, 17, 19. In some cases the cavity is so much reduced that it becomes inconspicuous.

Fourth Cleavage.—The fourth cleavage marks an interesting epoch, as it is the first to take a direct part in the formation of small cells at the animal pole. For the complete delimitation of the cells another cleavage is necessary, namely, a horizontal cleavage which cannot be seen from the surface and which affects only the eight cells bounded superficially by the fourth cleavage grooves, or by what we may call the circular groove. The latter usually appears midway between the pole and the margin of the calotte. It represents, strictly speaking, eight distinct grooves, one for each of the eight primary segments, but these usually run together in such a way as to form a continuous groove, encircling the pole and bounding a polar field which may have a circular form (Figs. 6, 25, 26) or a more elongated oval form (Figs. 16, 18). In one egg (Fig. 6) we have traced the order and direction of these grooves, noting also the time and place of origin as indicated in the figure.

There are many variations in this cleavage. It often happens that the eight constituent grooves run at various angles and are discontinuous, so that the polar field is bounded by a more or less irregular or zigzag line (Figs. 16, 18); sometimes one or more of these grooves take a meridional direction, as that shown in the upper part of Fig. 18.

This circular groove looks externally as if it might actually cut off polar segments, but as an examination of sections (Cuts 6, 7, 13, and 15) will show, the groove descends more or less obliquely, or centripetally, so that when completed its inner deeper boundary would be lost in confluent vacuolar spaces. As the plane of the groove probably never passes below the vacuoles, one might say that it describes an axial mass in the upper hemisphere approximating in shape an inverted truncated cone. Cut 6 is taken in the plane of the dotted line in Diagr. E. The circular groove appears to cut off two cells; this appearance is due to the section passing near the margin of the polar field. In Cut 7, which passes parallel with the second cleavage (plane of dotted line, Diagr. F.), these cells are still continuous with the yolk below. Cuts 13 and 15, taken along the dotted lines of Cut 7, further illustrate this point. It will be seen that at their bases the segments lying within the fourth cleavage grooves (IV) are likewise continuous with the yolk. Cut 8, taken at the level of the dotted line in Cut 7, shows the central grouping of the vacuolar spaces and the depth of the cleavage grooves at this time.

Dean (No. 3, p. 427, Fig. 24) figures and describes the "central blastomeres" of this stage as "separate from the underlying germ-yolk." In describing the next cleavage we shall explain how the central cells are cut off.

Fifth Cleavage.—The fifth cleavage comprises two distinct sets of grooves. One set appear on the surfaces of the eight primary segments in the form of meridionals. In Fig. 26 they are just beginning, and are more advanced in Fig. 27. The other set extend horizontally in the eight cells of the polar field, and of course are not visible from the surface. This cleavage thus brings about the 32-cell stage, and makes the central portion of the calotte two cells deep. The origin of yolk cells is thus not limited to the margin of the calotte, as in pelagic teleost eggs, but extends to all the cells beneath those of the polar field. These cells go on budding off cells to the germ-disc. It is rather interesting to see this horizontal cleavage appearing with the passage from the 16 to the 32-cell stage, because in the corresponding stage in the pelagic fish egg it

EXPLANATION OF CUTS 7 TO 12.

CUT 7.—Vertical section of the same egg as that from which the section shown in Cut 6 was taken. Its position (dotted line in Diagr. F) is much nearer the centre of the egg. The section shows that the cells apparently cut off in Cut 6 are continuous with the yolk, the oblique character of the fourth (IV) cleavage grooves, and the elongated nuclei of the central cells.

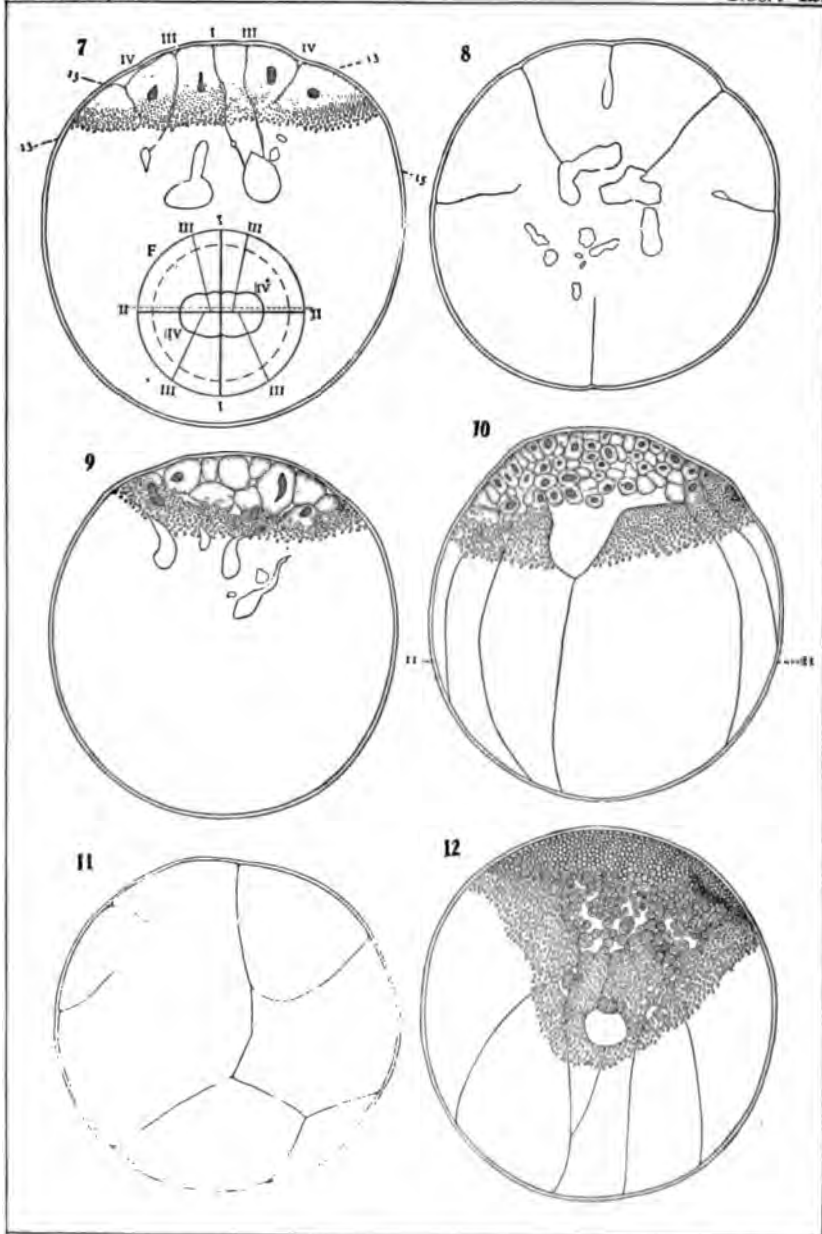
CUT 8.—Horizontal section of an egg in the same stage, in the plane indicated by the line 8-8 shown in Cut 6. It shows the central grouping of the vacuoles, and the depth to which the grooves have at this time cleft the yolk.

CUT 9.—Vertical section of an egg in the stage shown in Fig. 8, Pl. XVIII. The central cells have been cut by both horizontal and vertical grooves. Nuclei are present in the yolk.

CUT 10.—Vertical section of an egg in the stage of early blastula, showing the cleavage cavity and the extent to which the yolk is now segmented.

CUT 11.—Horizontal section of an egg in the same stage, in the plane of the dotted line 11-11 in Cut 10.

CUT 12.—Vertical section of an egg in a late blastula stage. The yolk has now begun to extend marginally over the yolk, and the epiblastula layer is beginning to differentiate.



takes effect only in the four central cells of the 16-cell stage. The marginal cells are divided by verticals which correspond to what happens in the eight yolk segments of the *Amia* egg.

Variations in this cleavage are numerous. It often happens that one or more of the central cells are cut by grooves passing parallel with the circular. Again, some of the grooves, instead of taking a horizontal or a circular form, may pass vertically, as indicated both by surface views and the elongation of the dividing nuclei. Whether the grooves cutting the central cells shown in Fig. 7 are to be interpreted as variations, or whether they represent a part of the sixth cleavage, we are unable to say. In this egg the central grooves appeared shortly after the appearance of the grooves dividing the marginal cells, with which they soon became continuous.

Sections of this stage are shown in Cuts 9, 14, 16, and 18. In Cut 14 the plane of elongation of the nuclei in the large marginal cells confirms what has been described from surface views. In Cuts 16 and 18 the elongation of the nuclei (V) lying within the circular groove (IV) shows that the next division of these cells will take place in a horizontal plane. While this is undoubtedly the usual plane of division, there are exceptions. In each of the sections shown in Cuts 14 and 15 one cell of the eight delimited by the fourth cleavage shows its nucleus elongated in such a direction that the resulting cleavage will be circular. In other sections the elongation of the nuclei indicates that vertical cleavages may also replace the horizontal. Cut 9 represents a vertical section of an egg in the stage shown in Fig. 8. The horizontal cleavage has evidently taken place, and in addition to this another set of verticals have appeared. In this section it was impossible to trace the cleavage grooves through the yolk.

Later Cleavage. — The next cleavage, which might be called the sixth, is shown in Figs. 8 and 28, and consists in a general way of two sets of circular furrows, one of which appears between the first circular and the upper pole, the other between the first circular and the margin of the calotte. In addition to these, new verticals have arisen in some of the marginal segments.

Figs. 29 and 30 represent eggs of about the same stage as that shown in Fig. 28. In Fig. 29 a radial symmetry is noticeable in the arrangement of the smaller cells, while in Fig. 30 a bilateral grouping is evident. Fig. 31 represents a third egg in about the same stage, showing the position and extent of the furrows, which at this time have reached the vicinity of the lower pole. A somewhat later stage is shown in Fig. 32.

Cut 10 represents a vertical section of an egg about 10 hrs. after deposition. The calotte is now from four to six cells thick. Beneath the central portion is the segmentation cavity, which at this time has become greatly enlarged through the confluence of the vacuolar spaces. In some eggs (*e.g.*, Figs. 18, 19) at this stage the cavity is so much enlarged that it appears like that seen in Amphibian eggs. The floor of the cavity is made up of large yolk segments. A horizontal section (Cut 11) of the same stage taken in the plane of the dotted line of Cut 10 shows the number and depth of the grooves at this level; above this level, near the equator, the grooves are more numerous.

Cut 12 represents a vertical section of an egg 35 to 40 hrs. after deposition (late blastula). The calotte, which has now begun to extend over the yolk, consists of thickly crowded spherical cells which marginally pass abruptly into the large yolk segments, while in the central portion they gradually increase in size and lie loosely scattered. The outer layer of the calotte is distinctly differentiated in that the cells are elongated and more densely granular. The entire yolk is irregularly cleft, the cells forming the lower portion are roughly polygonal and grade off into the large yolk spheres which lie at the centre.

THE RELATION OF THE EMBRYO TO THE FIRST TWO CLEAVAGE PLANES.

The elongated form of the egg of *Amia*, in a closely applied envelope, prevents rotation about its minor axes. It is therefore a favorable egg for ascertaining what effects, if any, gravity

EXPLANATION OF CUTS 13 TO 19.

CUT 13.—Oblique section of an egg in the stage of the fourth cleavage, just before the fifth cleavage. The plane of the section is represented by the dotted line 13-13 in Cut 7. The section shows on one side that the fourth cleavage has not yet cut off the central cells.

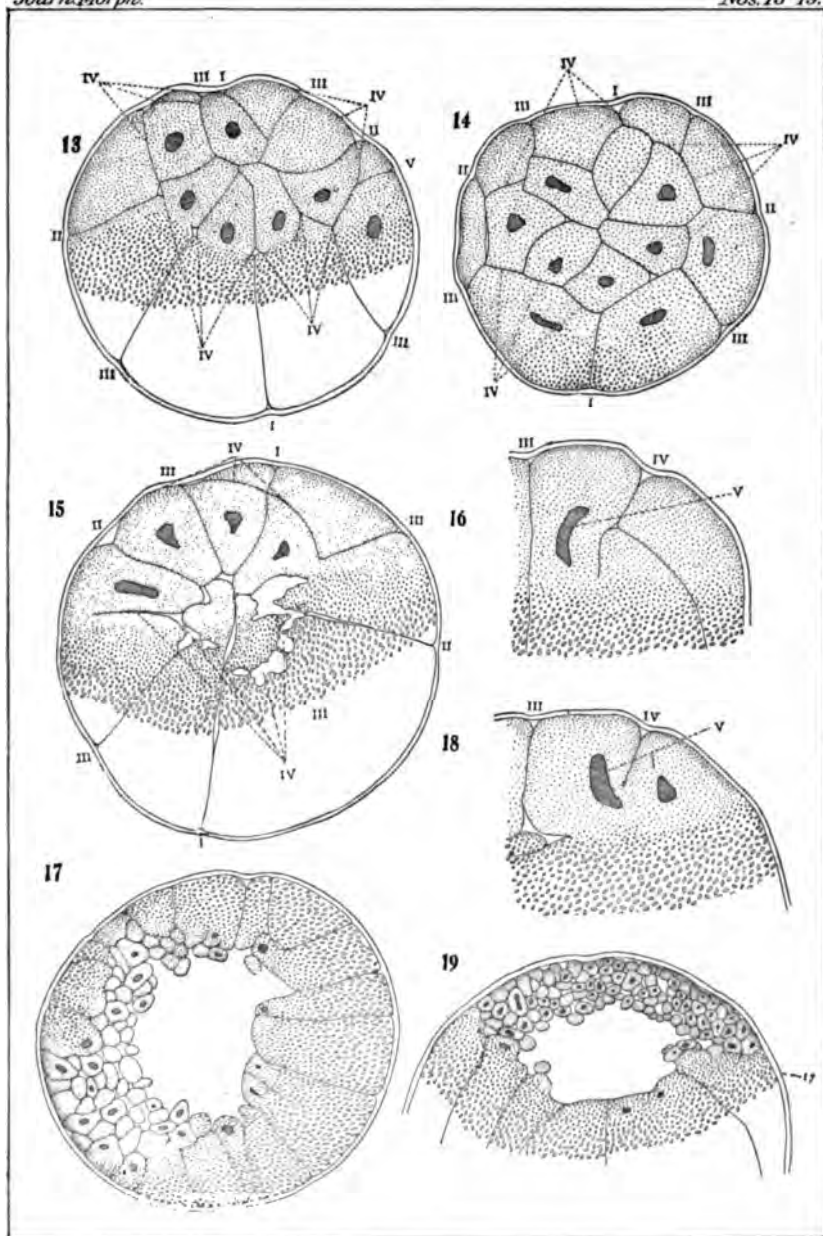
CUT 14.—Oblique section of the same stage, passing along the line 14-14 of Cut 6. The section shows the plane of elongation of the nuclei in the marginal segments which are soon to be divided by a set of verticals, forming a part of the fifth cleavage.

CUT 15.—Oblique section along the line 15-15 of Cut 7. The section shows that the circular groove (IV) becomes continuous below with the vacuolar spaces.

CUTS 16 and 18.—Vertical sections of the calotte from different eggs in the stage of fourth cleavage. The sections show the vertical elongation of the nuclei of the central cells preparatory to the horizontal cleavage which is to divide them.

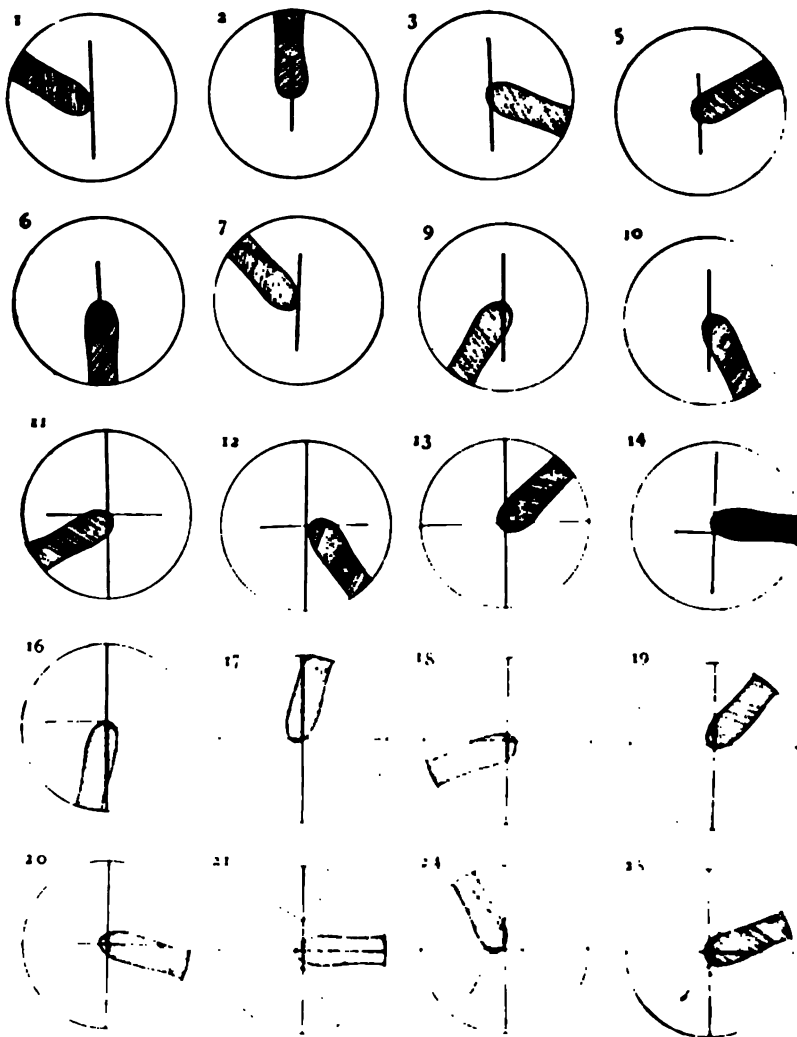
CUT 17.—Oblique section of an egg in a stage a little earlier than that shown in Cut 16. The section passes in the plane indicated by the line 17-17 in Cut 16. The section shows an exceptionally large oblong cavity. It also shows numerous yolk nuclei lying at the inner ends of the large yolk segments.

CUT 19.—Vertical section of a typical blastula. The cleavage cavity of this egg is also exceptionally large. Some of the large yolk segments may be seen dividing at their inner ends, the cells thus derived being continually added to the calotte.



may have on the direction of cleavage, and for determining the relation of the early cleavage planes to the median plane of

20



the embryo. It can be seen from 1-20, from the early stages of segmentation, that the cleavage planes are not in the normal way when the position of the egg was reversed. This is true

not only for inverted eggs, but for eggs placed in any position whatsoever. It seems to follow that gravity can have no directing influence on the cleavage.

In order to ascertain whether there is any constant relation of the embryonic axis to either of the first two cleavage planes, eggs were fixed in given positions by weighting, as before mentioned, and a sketch of the early grooves was carefully made in each case. These grooves are easily identified for a long time in the lower hemisphere of the egg, even as late, in some cases, as the early stages of gastrulation. As the sketches made at successive intervals showed no movement of the egg during all this time, it seems probable that the position of the egg remained practically unchanged up to the time when the median plane of the embryo was ascertainable. In some cases accidental markings on the surface of the egg remained in a fixed position until the embryo was well defined.

Our observations were made on three sets of eggs. The results are tabulated below and illustrated by the diagrams shown in Cut 20.

First Series. — At 8 A.M. May 12, 1895, ten eggs in which the first grooves had just appeared were fixed in position and sketches made at successive intervals. Two of the eggs died during gastrulation, leaving eight in which the embryos were apparently normal. The results were as follows :

EGG.	EMBRYO VISIBLE.	ANGLE WITH FIRST GROOVE.
1	6 A.M. May 15	67°
2	6 A.M. "	0°
3	5 A.M. "	75°
4	Died during gastrulation	
5	5 A.M. May 15	75°
6	5 A.M. "	0°
7	5 A.M. "	45°
8	Died during gastrulation	
9	5 A.M. May 15	30°
10	6 A.M. "	25°

Second Series. — At 9 A.M. on the same day, a second lot of ten eggs in which the second grooves were well under way were fixed in position. Nine of these formed normal embryos.

Egg.	EMBRYO VIBRILE.	ANGLE WITH FIRST GROOVE.
11	5.30 A.M. May 15	70°
12	5 A.M. "	35°
13	5 A.M. "	45°
14	5 A.M. "	88°
15	Died in late cleavage	
16	5 A.M. May 15	4°
17	5 A.M. "	4°
18	5 A.M. "	70°
19	5 A.M. "	35°
20	5 A.M. "	85°

Third Series.—At 10.30 A.M., on the same day, a third lot of five eggs, in which the third set of grooves had begun, were fixed in position. Only three of these formed embryos.

Egg.	EMBRYO VIBRILE.	ANGLE WITH FIRST GROOVE.
21	6 A.M. May 15	90°
22	Died	
23	Died	
24	5 A.M. "	30°
25	5.15 A.M. "	70°

The results may be summarized as follows:

1. Angles formed by median sagittal plane of embryo and first cleavage plane:

- In 10%, coincidence.
- In 10%, less than 5°.
- In 20%, greater than 5° and less than 45°.
- In 10%, 45°.
- In 35%, greater than 45° and less than 85°.
- In 10%, greater than 85° and less than 90°.
- In 5%, 90°.

2. Angles formed by the median sagittal plane of the embryo and the second cleavage plane:

- In 5%, coincidence.
- In 10%, less than 5°.
- In 35%, greater than 5° and less than 45°.
- In 10%, 45°.
- In 20%, greater than 45° and less than 85°.
- In 10%, greater than 85° and less than 90°.
- In 10%, 90°.

CONCLUDING REMARKS.

Dr. Dean (No. 3, p. 425) states that he "*has taken especial care to verify his observations on the meroblastic character of the cleavages of Amia. During the first cleavages several hundred living eggs were examined, with a view of determining holoblastic variations. These, however, did not occur, nor were there found, even by the most favorable means of illumination, traces of what might be construed as surface furrows traversing the yolk region of the egg. In no case did a marginal cleavage pass below the rim of the germinal disc.*"

If an author can say all this of an egg that is plainly holoblastic, how can we accept his testimony against Balfour, to the effect that the egg of *Lepidosteus* is meroblastic? If this egg be holoblastic, its cleavage might be said to agree very closely with that of *Amia*. It would be of interest to know when the first horizontal cleavage occurs. Up to the 8-cell stage the cleavage grooves may have practically the same order and arrangement in *Amia*, *Lepidosteus*, *Acipenser*, and the teleost. In passing to the 16-cell stage we usually get four central cells, inclosed by twelve marginal ones in the teleost. This arrangement, according to Dean's figures, holds both for *Lepidosteus* and *Acipenser*. In *Amia* we get ordinarily the circular groove, resulting in eight small cells in the polar (central) field, surrounded by eight large cells. Thus far the three eggs agree in having no horizontal cleavage. The passage to the 32-cell stage introduces, in both *Amia* and the teleost, the first horizontal cleavage; but in the former we have eight cells dividing in this way, in the latter, only four. The resemblances in other respects would lead us to expect this form of cleavage at the corresponding stage in *Lepidosteus* and *Acipenser*. In that case these ganoids would agree with the teleost in having four central (polar) cells thus divided.

In the more regular forms of holoblastic cleavage, as is well known, the first horizontal, or the equatorial, as it is often called, comes in as the third cleavage. Is this cleavage, when it occurs as the third, homologous with the horizontal, which appears as the fifth in the teleost, *Amia*, and probably other

ganoids? How are we to determine homology in such a case? To suppose, as some authors have done, that the first horizontal cleavage of the frog's egg is skipped in the case of the fish egg, is nonsense. There is no skipping in cleavage so long as the division of the cytoplasm follows regularly the order of nuclear division. If nuclear division runs on unaccompanied by segmentation of the egg, as in the insect egg, then we may speak of skipping cleavage; if cleavage does not appear until after three or more nuclear divisions, and then begins and takes the regular course, splitting into two, four, eight, etc., as in the crab's egg, according to Paul Meyer, we may speak of deferred cleavage. In the fish egg the cleavage is neither deferred nor skipped. Where, then, is the homologue of the third cleavage of the frog's egg? If position and relation to the axes of the future embryo are to decide homology, then there is no homologue before the fifth cleavage, and no certainty of any even then. To assume homology between a cleavage of the 4-cell stage and one of the 16-cell stage is to venture into a hopeless tangle of anachronisms. If order of succession is to be our criterion, then first is first, second is second, third is third, and so on, without regard to the position of the planes of cleavage or the fate of the cells. In either case, the attempt to homologize cleavages leads to contradictions and confusion. Homology that means nothing beyond the second cleavage, or that may fail even at the first cleavage, is its own negation. The wide range of variation in cleavage in many eggs, its total suppression in many others, and the possibility of changing its course by artificial means, without affecting the final result, all go to show, as it seems to us, that homologies do not depend upon cleavage planes. Even the first and second cleavages, which appear, at first sight, to agree so closely in widely different eggs, may cross the future lines of homology at various angles. If there is any homology in cleavages worth talking about, it must be because they take definite and constant parts in defining homologous organs or areas. Something of this sort has been claimed for the first cleavage. On further examination the claim proves to be ill founded. Often, as early as the 8 and 16-cell stages, the first groove is transformed into a

zigzag line, running irregularly, now to one side, now to the other, of the median plane.

If homologies do not depend upon the form of cleavage, and may even unfold without any cleavage at all, how are we to explain the fact that in many cases (*e.g.*, annelids, molluscs) cleavage is so constant, and so early becomes coincident with lines of homology? What determines this coincidence? This question has been discussed by one of us elsewhere, and we need not repeat here the conclusions reached.

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EXPLANATION OF PLATE XVIII.

All figures, excepting 8, 14, 17, and 20, drawn from living eggs. The numerals affixed to the cleavage grooves give the time in hours and minutes at which the grooves reach the points indicated by the dotted lines. The egg shown in Figs. 3-8 remained in a constant position.

Figures 9-20 are so arranged that the first cleavage groove has always the same position.

FIG. 1. Unsegmented eggs attached to grass. Natural size.

FIG. 2. Profile view of the unsegmented egg, showing natural color of the egg and the villi by which it is attached. Made by J. Nomura. $\times 15$.

FIG. 3. Profile view of the egg 3 hrs. 23 min. after deposition, showing position and extent of the first vertical groove. $\times 14$.

FIG. 4. Profile view of same egg at 4 hrs. 19 min. The second verticals have extended slightly beyond the margin of the calotte. $\times 14$.

FIG. 5. Profile view of the same egg at 5 hrs. 10 min., showing the position and extent of the first three sets of verticals. $\times 14$.

FIG. 6. Profile view of the same egg at 6 hrs. 20 min., after the appearance of the circular groove. $\times 14$.

FIG. 7. Profile view of same egg at 7 hrs. 23 min. The fourth vertical cleavage is in progress. $\times 14$.

FIG. 8. Profile view of same egg at 8 hrs. 25 min., showing additional circular grooves, one set within, the other without, the first circular groove. $\times 14$.

FIG. 9. Showing a case in which the calotte is unequally divided by the first groove. $\times 13$.

FIGS. 10, 11, 12. Views of the upper pole, showing variations occasionally observed in the position of the second verticals. $\times 13$.

FIG. 13. View of the upper pole, showing variation in position of one of the third verticals. $\times 14$.

FIG. 14. View of the lower pole of same egg. $\times 13$.

FIG. 15. View of the upper pole, showing another variation in the position of the third verticals. $\times 13$.

FIG. 16. View of the upper pole, showing a variation in the position of the grooves of the first circular cleavage. $\times 13$.

FIG. 17. View of the lower pole of the same egg, showing points at which the second verticals terminate. $\times 13$.

FIG. 18. View of the upper pole, showing a broken circular groove. $\times 13$.

FIG. 19. View of the upper pole of an egg in a stage somewhat later than the one shown in Fig. 8. $\times 13$.

FIG. 20. View of the lower pole of same egg. $\times 13$.

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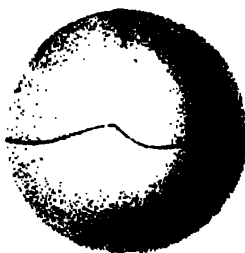
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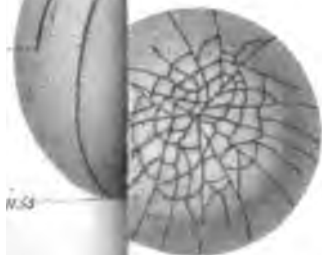
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EXPLANATION OF PLATE XIX.

All the figures were drawn from material fixed in chrom-osmic and preserved in 80% alcohol. Magnification about 16 diameters.

FIG. 21a. View of the upper pole of the egg, showing the position of the first groove and micropylar orifice.

FIG. 21b. Profile view of the same egg.

FIG. 22a. View of the upper pole of the egg at the beginning of the second vertical cleavage.

FIG. 22b. Profile view of the same.

FIG. 23a. View of the upper pole, showing symmetrical third verticals.

FIG. 23b. Profile view of the same.

FIG. 24a. View of the upper pole in same stage as Fig. 23, showing an asymmetrical position of one of the third verticals.

FIG. 24b. Profile view of the same egg.

FIG. 25a. The formation of the first set of circular grooves.

FIG. 25b. Profile view of the same.

FIG. 26a. The first set of circular grooves completed, and the fourth set of verticals beginning.

FIG. 26b. Profile view of the same.

FIG. 27a. Fourth set of verticals well advanced.

FIG. 27b. Profile view of the same.

FIG. 28a. Shows the addition of two new sets of circular grooves, one within the other without the first one being lost.

FIG. 28b. Profile view of the same.

FIG. 29a. A little later stage than Fig. 28, but not yet quite as far advanced as those seen in Fig. 28.

FIG. 29b. Profile view of the same.

FIG. 30a. About the same stage as Fig. 29, but with a different arrangement.

FIG. 30b. Profile view of the same.

FIG. 31a. The lower pole of the egg in same stage as Fig. 30.

FIG. 31b. Profile view of the same.

FIG. 32a. Another view.

FIG. 32b. Profile view of the same.

ON THE MODES OF DEVELOPMENT OF THE MESODERM AND MESENCHYM, WITH REF- ERENCE TO THE SUPPOSED HOMOLOGIES OF THE BODY CAVITIES.

THOS. H. MONTGOMERY, JR., PH.D.

MESODERM is the term broadly applied to that embryonic cell mass situated between the primitive ecto- and entoderm. The mesoderm, *i.e.*, mesodermal cells, and not the extracellular gelatinous substance secreted in the archicoel by the ecto- and entodermic cells, is derived either from one of these two layers or from both of them. It is derived (1) from the ectoderm in the Anthozoa, Porifera; (2) from the entoderm in Ctenophora, Polycladidea, Nemertini (in part), Nematodea, Annelida, Hirudinea (?), Sipunculacea, Chaetognatha, Balanoglossus, Echinodermata, Copepoda, Insecta (?), Mollusca, Phoronis, Ectoprocta, Brachiopoda, Entoprocta (?), Tunicata, Amphioxus; (3) from both ecto- and entoderm: in the Nemertini (Desor's type), also perhaps in the Rotatoria and many of the Arthropoda (except Copepoda, Insecta). The origin of the mesoderm from the entoderm is thus the most usual.

The mesoderm may arise from the entoderm in three ways: (1) by multipolar delamination; (2) by unipolar delamination; (3) in the form of epithelial diverticula.

(1) The multipolar delamination, the so-called mesenchym production (Mesenchymbildung), consists in the separation of cells from the entodermal layer at various points on the inner surface of the latter, which become situated in the archicoel, in the gelatinous substance secreted by both ecto- and entoderm. Such mesenchym production is typical for the Nemertini, Echinodermata, and Ectoprocta.

(2) The unipolar delamination of the mesoderm from the entoderm takes place through the so-called pole-cells of the mesoderm, *i.e.*, one or two cells which divide off from the entoderm at one pole of the latter, and by the cleavages of which

the mesodermic cell layer is produced. These pole-cells always lie in the archicoel. Such a production of the mesoderm is typical for the Annelida and Mollusca; further, in more or less modified form, for the Nematodea, Copepoda, and Entoprocta.

(3) Finally, the mesoderm may be produced from the entoderm, in the form of epithelial diverticula, or the so-called coelom sacks. Such coelom sacks may be (a) evaginations of the gastrocoel, or (b) invaginations into the gastrocoel.

(a) The mesoderm is produced in the form of a coelom sack, which is an evagination of the entoderm, and the coelomic cavity of which is consequently a derivative of the gastrocoel: in the Echinodermata (the vasoperitoneal vesicle), Balanoglossus, Brachiopoda, Ascideae, and Amphioxus.

(b) The mesoderm is produced in the form of a coelom sack, which is an invagination of the entoderm, and the coelomic cavity of which is therefore derived from the archicoel, in Sagitta and Phoronis.

The three types of development of the mesoderm delineated above are not sharply distinct, since they differ from one another rather in degree than kind, and are connected by intermediate stages. Thus we read on p. 12 of Korschelt and Heider's *Lehrbuch*: "So verschieden diese beiden Arten der Mesodermbildung (nämlich Urmesodermzellen, Urdarmdivertikeln) auch scheinen mögen, so lassen sie sich doch auf ein einheitliches Schema zurückführen, wenn wir annehmen, dass im ersteren Falle die Mesodermdivertikeln frühzeitig (als Urmesodermzellen) den epithelialen Verband des Entoderms verlassen, während im zweiten Falle die Mesodermzellmasse vorläufig in dem epithelialen Verbande bleibt und erst später durch die Divertikelbildung zur Lostrennung gebracht wird" (*cf.* also p. 267). In fact, I would refer both the production of the pole-cells and of coelomic diverticula to the more primitive mode of formation of multipolar mesenchymatic migration. By the mesenchym cells remaining in contact as an epithelium, coelomic diverticula are produced, which are bipolar but multicellular in origin and character. In the case of the two "Urmesodermzellen," I would explain, in agreement with Korschelt and Heider, the two pole-cells as two mesenchym

cells, the mesenchym tissue being here unipolar and bicellular, instead of multipolar and multicellular in origin.

Further, in meroblastic ova, from which the gastrulae are not of the invagination type, but sterroglastulae, etc. (as in the Cephalopoda, Sauropsida, Hirudinea, Turbellaria, most Arthropoda, etc.), the whole process of production is so much modified by the amount of yolk in certain of the blastomeres, that the mode of development of the mesoderm can with difficulty be referred to the three more primitive modes of development given above.

The examples given show how multifarious the development of the mesoderm is in different animal groups.

Again, there is no sharp distinction between mesenchym and mesoderm, but only a difference of degree; as is shown in a large number of the newer embryological researches. Thus, while the term "mesenchym" is generally applied to cells of the third primitive embryonic layer which are not united together in a continuous mass, and while "mesoderm" is applied to cells of that layer when from the beginning they are in contact with one another, forming a mass of cells ("mesoderm-stripe"), we find, in the case of the production of the mesoderm through typical pole-cells, that the two cells may be or may not be in contact, but that the mesoderm cells proliferated by each construct a solid cell mass. In fact, whether the cells of the third embryonic layer are not united ("mesenchym") or are united ("mesoderm," *sensu strictiori*) would seem to depend upon two factors: (1) the comparative size of the archicoel and (2) upon the modes of production of the third embryonic layer, which modes we have found to intergrade. And in such groups as the Mollusca and Arthropoda, it is difficult to decide whether the third embryonic layer is to be classed as a "mesoderm" or as a "mesenchym." Accordingly, these two terms express but extremes of a series, and morphologically cannot be sharply distinguished.

Lastly, as to the morphological value of the cavity enclosed by the mesoderm cells. This cavity is a derivative of the archicoel in all those forms where the mesoderm is formed (1) by multipolar, mesenchymatic delamination, or (2) by pole-cells,

or (3) by invaginated diverticula of the entoderm (in *Sagitta* and *Phoronis*). It is a derivative of the gastrocoel when the coelomic sacks are evaginations of the entoderm (as in the *Echinodermata*, *Balanoglossus*, *Brachiopoda*, *Ascideae*, and *Amphioxus*). In the *Ctenophora* it is both gastrocoelic and archicoelic in origin. This cavity is called a pseudocoel when it is not lined by a continuous epithelium of cells, and a coelom when it has such a lining. The pseudocoel of a turbellarian and the coelom of an annelid are both derivatives of the archicoel; but the coelom of *Amphioxus* is gastrocoelic in origin. In the same animal, coelom and pseudocoel, when both cavities occur together, may communicate. From these facts we may conclude, that there is also no valid morphological distinction between a pseudocoel and a coelom, when both are derivatives of the archicoel. Whether, however, a morphological distinction can be drawn between the archicoelic coelom of an annelid and the gastrocoelic coelom of an *Amphioxus*, is a question which I believe has not yet been considered.

In regard to the ontogenetic derivation of the blood-vessels, or blood lacunae, their endothelia, and the free blood corpuscles, the following points are of interest. These observations are based mainly upon data given in the zoölogical text-books of Korschelt and Heider, Hatschek, and Arnold Lang.

In the Nemertini the cavities of the blood-vessels and of the rhynchocoel are archicoelic, and these are lined by endothelia, from which the floating cells are derived. And, as I have shown in a paper, "On the Connective Tissues and Body Cavities of the Nemerteans," etc., to appear in Spengel's *Zool. Jahrb.*, the gonads and their products are also archicoelic—at least in all those forms in which the gonadal sacks are then first formed, when the sexual cells become differentiated from the somatic cells.

In the Chaetopoda and the Archiannelida the cavity of the blood-vessels is archicoelic, their walls, from which the free corpuscles are derived, splanchnopleuric in origin. In the Hirudinea, to cite Korschelt and Heider (p. 222): "Von den dorsalen und ventralen Blutgefäßstämmen ist angegeben worden, dass sie vom splanchnischen Blatt aus, durch Spaltung dessel-

ben, ihren Ursprung nehmen"; they stand in open communication with the pseudocoelic body sinuses, the latter having an endothelial lining in the Rhynchobdellini, but not in the Gnathobdellini. The blood corpuscles of the leeches are probably derived from the blood-vessels as well as from the so-called "parenchym" (Lankester). In the Sipunculacea, there is probably a communication between the blood-vessels and the coelom, the splanchnopleuric peritoneum of the latter being ciliated, as in the frog (Lang, p. 213).

Echinodermata: "Spaltbildungen im Mesenchym sollen die Blutgefäße der Holothurie entstehen lassen. . . . Die Blutzellen hingegen sollen sich von den Wandungen des Hydroenterocoels losgelöst und bei der Bildung jener Gefäße beteiligt haben. Diese freien Zellen, welche sich sowohl in der Leibeshöhle, wie in den Ambulacral- und Blutgefäßen finden, würden also nach dieser Auffassung (Semon) nicht von dem ursprünglichen Mesenchym abstammen" (Korschelt and Heider, p. 286). In the Asteroidea: "In der zwischen Hydrocoel-, Enterocoel-, und Darmwand gelegenen Mesenchymschicht bildet sich dort ein Spalt, welcher eine Auskleidung von sehr flachen Zellen aufweist" (*ibid.*, p. 290). The blood-vessels in the Crinoidea communicate with the coelom, and arise as cavities in proliferations of the enterocoel (*ibid.*, p. 302).

In all the Arthropoda, as is well known, the blood-vessels stand in communication with the body cavities. The heart of the Crustacea is mesodermal in origin, its cavity archicoelic (Korschelt and Heider, p. 376). In *Limulus* (*ibid.*, p. 528), the heart develops from the mesodermal plates, and cells wander into its cavity, which had their origin in its walls. In the Scorpionidea (*ibid.*, p. 556), a segmented coelomic cavity arises in the primitive mesodermal proliferation, the walls and free corpuscles of the blood-vessels being mesodermal (is their cavity then coelomic?). As to the Araneina (*ibid.*, p. 615), it is a disputed point whether the blood corpuscles are merocytes or whether they originate from the protovertebrae; later they form a solid chord on the dorsal side of the embryo; they separate from one another again, when the heart becomes formed by a coalescence of neighboring splanchnopleuric layers; thus the

cavity of the heart is archicoelic. In *Peripatus* (*ibid.*, p. 711), the heart is formed by mesodermal cells, its cavity being archicoelic. The blastocoel of the *Myriapoda* (*ibid.*, p. 752) becomes filled with yolk, while later mesodermal cells penetrate and surround the yolk, producing a pseudocoel; these cells accomplish the formation of the blood-vessels, and perhaps also of the heart. The body cavity of the *Insecta* is a product of the primitive archicoel and the coelom; the heart is formed of mesodermal cells of protovertebral origin, and the blood corpuscles are also of similar derivation (*ibid.*, pp. 818, 833, 834).

In the *Mollusca* the archicoelic (pseudocoelic) body cavity: "stellt im Allgemeinen das Lacunen- und Sinussystem des Körpers dar, in welches sich die Arterien öffnen, und aus welchem die Venen, wo solche vorhanden sind, ihr Blut beziehen. Sie ist ohne eigene Epithelwand" (Lang, p. 792). The true coelom is much reduced, being represented only by the pericardial and gonadal cavities, and is bounded by its own epithelium. The blood-vessels (a closed system only in the *Cephalopoda* and certain *Prosobranchs*) have no endothelium (Lang, p. 780). In the *Lamellibranchiata*, the heart, aortae, and gillveins are produced by the agency of mesodermal cells in the primitive body cavity; there is no communication between the blood-vessels and the pericardial cavity (Korschelt and Heider, pp. 971, 973). The heart in the *Gasteropoda* is derived from the pericardium, while "die Gefässe entstehen als Lückenräume im mesodermalen Zellmaterial der primären Leibeshöhle (Blastocoel), also zunächst ganz unabhängig vom Herzen" (*ibid.*, pp. 1081, 1082). In *Phoronis* a coelom is well developed and is bounded by an endothelium (Lang, p. 213). The blood-vessels probably arise as lacunae in the splanchnopleuric mesoderm: "während nach Cori das Gefäßsystem des ausgebildeten Thieres ein vollständig geschlossenes ist, scheint in der Larve eine Communication zwischen demselben und dem Kopftheil der Leibeshöhle zu existiren. Im letzteren sollen die Blutkörperchen in Massen angehäuft ihre Entstehung nehmen" (Korschelt and Heider, p. 1184).

In the *Ascideae*, the early coelomic cavities give way later to a mesenchym, which fills the archicoel: "die später in die-

sem Mesenchym auftretenden Lacunen müssen ebenso wie die Blutgefäße (welche . . . einer endothelialen Wand vollkommen entbehren) als Pseudocoel betrachtet werden. . . . Indem einzelne Zellen des Mesenchyms frei werden und in das Pseudocoel gelangen, bilden sie sich zu Blutkörperchen um" (Korschelt and Heider, pp. 1289, 1290). In *Salpa* the heart is of mesodermal origin, and the vessels are lined with an epithelial intima: "die Blutgefäße entstehen anscheinend als Lückenräume innerhalb jenes gallertigen Bindegewebes, welches in späteren Stadien die primäre Leibeshöhle erfüllt" (*ibid.*, p. 1346).

In the Vertebrata the cavity of the blood-vessels is probably archicoelic and their walls mesenchymatic in origin; but the present views upon the derivation of the corpuscles and of the blood-vessels themselves are very conflicting.

To recapitulate in regard to the origin of the blood-vessels and blood corpuscles: the cavity of the blood-vessels (with the exception of the heart in the Gasteropoda) is apparently always archicoelic (blastocoelic), and never gastrocoelic nor coelomic. The blood corpuscles in most of the animal groups are derived from the endothelial lining of the vessels where such membrane is present, but where it is absent, from the surrounding connective tissue elements. Accordingly, since the walls of the blood-vessels may be mesenchymic (Nemertini?, Hirudinea, Asteroidea, Ascidae, Vertebrata?), or may be mesodermal (Annelida, Holothuroidea, Limulus, Scorpionidea, Peripatus, Insecta, Lamelli-branchiata), so the blood-vessels themselves may be either mesodermal or mesenchymic, or both (*e.g.*, Hirudinea).

The foregoing brief summary of our present knowledge on the development of the various body cavities of the Metazoa would lead to the conclusion, that particular differentiations of this cavity in one group cannot be safely homologized with similarly situated cavities in other groups. And the reason for this is not far to seek. For, in the first place, the very different modes by which the process of gastrulation takes place — a process which seems to become modified by the mechanical factors determining the previous cleavage of the egg — induces very heterogeneous formation of both archi- and gastrocoel.

Thus, though under the term "archicoel" is understood the cleavage cavity between the embryonic layers ectoderm and entoderm, it is in reality also equivalent to the space between any two neighboring blastomeres, as, *e.g.*, the cavity between two ectodermal or between two entodermal cells, or even between an ectodermal and an entodermal cell. Further, since this cleavage cavity is, in pre-blastula stages at least, in a direct communication with the outside (as is well seen in the cleavage of the Ctenophora and Tricladidea), its relation to the gastrocoel is found to be close. And in the very frequent, and perhaps most primitive, method of gastrulation, according to which the entodermal cells delaminate from the ectoderm and wander into the blastocoel, we find that the cavity enclosed by the later entoderm, and thus comparable to a gastrocoel, is, in fact, a portion of the earlier blastocoel. So, nearly all intergradations may be found between the cleavage illustrated by the triclad Turbellaria, where the blastomeres are at first isolated (with consequently a large and open cleavage cavity), and the sterroblastic cleavage exhibited, *e.g.*, by the polyclad Turbellaria or the Rotatoria, where the blastocoel is represented merely by clefts between adjacent blastomeres.

Gastrocoel and blastocoel are nothing more than spaces between or enclosed by the blastomeres, which, in early stages at least, communicate with the outside. The gastrocoel may be in certain cases a space lined by entodermal cells, divided off from the primitive blastocoel, or it may be an extraneous space bounded by such cells. In the same group of animals both modifications may occur (*e.g.*, in the Mollusca, the Crustacea, and Turbellaria).

The embryonic body cavities known as blastocoel and gastrocoel are, therefore, not morphologically distinct spaces, and only in certain cases (*e.g.*, typical invagination gastrulation) are they to be separated. For the mode of formation of both is apparently dependent upon such factors as the mechanical pressure of the yolk, etc. (other factors might well be at work, which we have failed to recognize), that is, dependent upon the process of cleavage; and the latter process, as is well known, may present great differences in closely allied forms (as in the

Crustacea or Turbellaria), and hence no high morphological value can be attributed to it.

Obviously then the mesoderm, as well as the spaces enclosed or penetrated by mesodermal elements, cannot be granted importance in morphological classification; which deduction is in accord with a number of recent investigations, that stand in opposition to the acceptance of the germ-layer theory. For the development of the mesoderm and its cavities is, in its turn, dependent upon the cleavage and gastrulation processes. Thus a mesodermal pseudocoel as well as a coelom are usually blastocoelic; but the coelom may be also gastrocoelic in origin. Similarly there is no essential difference between formation of the mesoderm by detached and isolated cells, and by coelom sacks or epithelially united mesoderm stripes.

Accordingly, I am led to conclude that the body cavities in different animal groups cannot be homologized merely on the ground of apparent similarity of development; for the earlier development and differentiation of these cavities must be referred, directly or indirectly, to the modes of cleavage and gastrulation, and the latter, as is well known, often differ widely in closely allied forms.

The coelom of a vertebrate is frequently spoken of as being homologous with that of an annelid, since it passes through an apparently similar development. Now without stating or in any way wishing to imply that the homology of the coelom in this case is not correct, I would emphasize the point that the similarity of development is itself not an adequate reason for the homology. This standpoint should seem justifiable to any one acquainted with the facts reviewed in this paper, which tend to show how various the formation of mesoderm and its cavities are in closely related forms.

Are, then, the body cavities possible of homologization? Comparison of the modes of early development shows that the ontogeny is of little value in this connection; but it might be thought that comparative anatomy could be of avail in the search for homologies.

But, though often spoken of as such, a "body cavity" cannot be considered an organ, equivalent, *e.g.*, to a brain or a

nephridium, but rather it must be compared to a system of organs, since a number of different functions are performed by it, and since it stands in a peculiar relationship to most, if not all, of the bodily organs. The whole origin, manner of differentiation, and extent of development of the body cavity, is to a great extent dependent upon the mutual positions of the organs as well as upon their degree of correlation. Therefore, any change which causes a difference in the relative positions of the organs must also effect some degree of change in the diversification of the body cavity.

The body cavity being essentially a space or system of spaces separating and penetrating the organs of the body, a common possession of these organs, cannot be correctly homologized, for the very fact that it is not comparable to any individual organ. It would be as difficult to satisfactorily homologize these cavities in different forms as to establish homologies between states of correlation, or between mutual arrangements of parts. And in case examples are required we may consider the group of the Nemerteans, for it was a study of the body cavities in this interesting group of worms which led me to make the present examination into the question of possible homologies.

In the Nemerteans, the body cavity occurs as (1) the rhynchocoel (the large space surrounding the proboscis); (2) the cavity of the blood-vessels; (3) the perivisceral space situated between the intestine and blood-vessels on the one hand and the body muscular wall on the other. The portions of the cavity mentioned under (2) and (3) are remnants of the cleavage cavity (*i.e.*, archicoelic in origin); but whether the rhynchocoel also is archicoelic has not yet been definitely settled. Now in a previous contribution, to which I have already referred, I compared the pseudocoel in this group to the coelom of the Annelids, and for the following reasons: the Nemertean pseudocoel—that space the existence of which has, until recently, been questioned—encloses in the adult worm true mesenchym tissue consisting of multipolar cells; it is from certain of these cells that the sexual cells are derived. Further, in all the species where the gonadal sacks are not preformed,—and this is the case in

the majority of forms, — pseudoepithelia of these primitive sexual cells arrange themselves in the form of paired and metameric sacks, which are then the gonads. Thus the comparison holds good, that in both Annelids and Nemerteans the sexual products are derived from the lining of the perivisceral body cavity. In the Metanemerteans the blood-vessels are completely closed, while in the lower Nemerteans they are (in the head region) in communication with the pseudocoelic spaces: in the former as in the Chaetopoda, in the latter as in the Hirudinea. Thus parts of the body cavity in the Nemerteans may be compared to a true coelom, other portions to a pseudocoel (archicoel). The great difficulty of determining the homology of the Nemertean body cavity is simply due to the fact that it unites characteristics of a coelom and a pseudocoel — formations which, according to the mesenchym theory of Hertwig, had formerly been supposed sharply distinguishable. So we find the difficulty is in reality owing to the fact of the impossibility of correctly homologizing such structures as body cavities; for not only may two (supposedly) different types of cavities be present in the same species, but the two are frequently so intermingled in it as to render their recognition almost impossible in our present state of knowledge. Thus we are not in position to state whether the Nemertean body cavity is of Turbellarian or of Annelidan character.

The general conclusion which I would maintain, then, is that body cavities in different animal groups cannot be safely homologized, either from the ontogenetic or from the comparative anatomical standpoint; though the latter method would seem to be, in this matter at least, more reliable than the preceding. The body cavity, whether as coelom or as pseudocoel, is not comparable to any single organ or set of organs, but must be considered as a structure of approximately equal economy to all the organs. And in its early formation and later differentiation probably most if not all of the organs take part, beginning with the blastomeres as the earliest. Accordingly, before seeking to homologize the body cavity, the morphological value of all the organs themselves must be learned, as well as the morphological value of their mutual topography.

In my search for the homologies of the coelom, or pseudo-coel, of the Nemerteans, a search which has led me to negative results, I have been obliged to exceed the limits which I had at first intended; but it is to be hoped that this brief critical consideration of the morphological value of body cavities in general may throw a new light on these structures.

Any one wishing to compare earlier views upon their nature may consult, outside of the text-books of Balfour, and of Korschelt and Heider, the following papers:

- BALFOUR. On the Structure and Homologies of the Germinal Layers of the Embryo. *Quart. Journ. Micr. Sci.*, 20. 1880.
- BÜTSCHLI. Bemerkungen zur Gastraeatheorie. *Morph. Jahrb.*, 9.
- HAECKEL. Die Gastraeatheorie, die phylogenetische Classification des Thierreichs und die Homologie der Keimblätter. *Jena. Zeitsch.*, 8.
- IDEM. Nachträge zur Gastraeatheorie. *Ibid.*, 11.
- HATSCHEK. Studien über Entwicklung des Amphioxus. *Arb. zool. Inst. Wien*, 4. 1881.
- HERTWIG, O. AND R. Die Coelomtheorie. Versuch einer Erklärung des mittleren Keimblattes. Jena, 1881.
- LANKESTER. On the Primitive Cell-layers of the Embryo as the Basis of Genealogical Classification of Animals, and on the Origin of Vascular and Lymph Systems. *Ann. and Mag. Nat. Hist.*, 11. 1873.
- IDEM. Notes on the Embryology and Classification of the Animal Kingdom, etc. *Quart. Journ. Micr. Sci.*, 17. 1877.
- RABL. Theorie des Mesoderms. *Morph. Jahrb.*, 15. 1889.
- WALDEYER. Die neueren Forschungen im Gebiet der Keimblattlehre. *Berlin. klinisch. Wochenschr.* 1885.

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY,
PHILADELPHIA, PA., July 15, 1896.

SOME SPINNING ACTIVITIES OF PROTOPLASM IN STARFISH AND SEA-URCHIN EGGS.

GWENDOLEN FOULKE ANDREWS.

THE observations recorded here were begun at the Marine Biological Laboratory of Wood's Holl, in the summer of 1893. For use of an "investigator's room" and other privileges enjoyed there for the third time, I am indebted to the kindness of the Director, Dr. Whitman.

While watching the protoplasm of developing starfish and sea-urchin eggs under very high powers, certain curious filose phenomena restimulated an interest that had for years concerned itself much with the "thread-forming," "filose," or, as I prefer to call them, the *spinning* activities of the living substance, especially as found among the Protozoa, where they are of widespread occurrence outside the extremely large group of protoplasts in which they are characteristic phenomena.

The eggs in which the supposedly typical phenomena were watched were normal, so far as could be discovered by a series of comparative observations and experiments on many other specimens, some of which developed normally into quite advanced stages. Great care was used to make little or no compression on these eggs, to keep the water fresh and plentiful about them, to maintain an even temperature, and to hold them but a few moments at a time under observation.

Other cases which were carelessly dealt with, or which were immature, or over-fertilized, showed plainly an abnormal state of their substance, which was visible in spinnings of a decidedly different character from those of the normal specimens. These cases were followed closely for long periods, and normal specimens were made abnormal by heat, or pressure, or confinement, so as to learn the peculiarities of such states, and, if possible, distinguish with regard to the spinning phenomena between these and the normal condition.

The observations at Wood's Holl were made with a Beck $\frac{1}{2}$ immersion, which had been carefully chosen from a number for its flatness of field, definition, and lack of color,—with apochromatic eyepieces.

At the Zoölogical Laboratory of the Sorbonne, Roscoff, Brittany, where I was enabled by courtesy of M. Lacaze Duthiers to make a series of similar observations, the instruments used were all of Zeiss,—largest size stand, with Abbé condenser and iris diaphragm ; 4.0 mm., and 2.0 mm. immersion, lenses ; with 4-12 apochromatic oculars.

While there proved to be nothing to correct in the first observations, they were much extended and amplified by the second series whose range of magnification brought to sight a greater length of filament, also secondary spinnings, or ramifications of these, and still other primary filaments far beyond the reach of the powers before used.

The sea-urchin eggs were from two genera ; those obtained at Wood's Holl, from *Arbacia* ; those used at Roscoff, from the common, large, and finely colored species of *Echinus* found along that coast.

The *Arbacia* eggs were perfectly normal in their initial conditions, and in large proportion developed to a late larval stage ; they, as well as the sperm, were obtained artificially from the animals. The *Echinus* eggs and sperm were also obtained artificially, but, being more hardy, gave, it was thought, for that reason more reliable data than *Arbacia* even, although the results in the one case but confirmed those in the other.

The starfish eggs used in both series of observations were of the same genus, but of different species. In all the test cases the eggs were laid and fertilized naturally in a tank shortly after capture. Those collected at Roscoff were sometimes from two or more females and two or more males depositing at one time. The eggs were, however, protected from too great admixture with sperm by instant separation, and afterwards carefully washed and then kept in small quantities in separate, large dishes in which the water was frequently changed.

In many of these dishes it was difficult later to find any eggs which had not reached a normal, free-swimming, larval state due at about that given time. The test specimens were kept isolated of course, but compared with results given by random specimens from the larger number.

In the eggs of *Arbacia*, I had seen the characteristic, striated membrane produced, immediately after entrance of the sperm, by formation of innumerable, delicate, thread-like processes from a clear pellicular layer of the egg. Although the resemblance between these threads and those formed in the *tuft* of protoplasm which receives the sperm was very great, I could not convince myself that they were not perhaps of non-living matter and caused by mere exudation of a glairy substance, which, if issuing from small, pore-like openings, could assume a thread-like shape. An appearance of partial return in single instances of the substance towards the egg, did not seem proof to the contrary, since it was thought that this would be quite possible were the substance of the nature suggested.

In the *Echinus* eggs, the question seemed to be solved by several cases of polyspermy, in which the egg, seen to be immature from the nuclear conditions, never formed a perfect membrane, but merely made abortive attempts to do so, the various portions of its surface having more or less success, or making, perhaps, more or less exertion, in *spinning*. Of the most typical instance of this sort, a camera lucida memorandum was made, showing the various protoplasmic activities of the pellicular substance, and also the several *tufts* which mark the entrance, complete or partial, of several sperm.

The whole surface of this egg, soon after entrance of the first sperm, was covered with a rather thick and albuminous-looking material, like ectosarcic protoplasm, in which no vesicular structure of Bütschli was visible, although there was a deceptive appearance of such, caused, as it seemed, by optical sections of papillose, or short, thread-like, processes.

This clear layer seemed at first to be only partially effective in preventing the entrance of more sperm, and it responded to the attempts of these with more or less pronounced tufts, and

showed wave-like modulations of its surface outline, possibly due to accession of more material from the egg.

About the point of entrance of the first sperm, there arose for some distance a host of delicate threads, closely resembling, except in their instability and their unevenness of length, those of the normal eggs. These threads had an optical effect of being outspinnings of the outer clear layer of the egg, and of the mere surface substance of this. At other regions there was sometimes an effect of the processes piercing this and arising from the inner or true surface of the egg itself. That they did arise in some places from the outer layer was shown by local formations of short, papillose roughenings of this, which were seen first as mere superficial unevennesses but which afterwards extended themselves so as to form true threads.

That the outer layer and its products were, for the time being at least, part of the living material of the egg, — a true, ectosarc-like formation, — seemed proven by ramifications of the threads in the more abnormal regions of spinning; by unstable anastomosis with each other of such secondary, and also of the primary, processes; and by the general flux of their substance to and from the layer, as well as amongst each other, in a manner most characteristic of protoplasmic phenomena. Such activities were noted in the tufts, and indeed there were no characters optically discoverable which enabled one to separate the two groups of phenomena in these abnormal eggs. In normal cases the single tuft is formed from a delicate covering of the egg, which has the same general character as the thick, abnormal envelope, being merely more stable and quiet.

At time of forming these areas and processes in the abnormal type-instance the nuclear membrane had not yet been dissipated, but lay somewhat to one side of the egg. The nuclear area, although perfect, showed itself to be immature by the mode of distribution of its elements.¹ The progress of this special egg

¹ Certain progressive differences which mark the development or maturation of this cell body are described in a forthcoming thesis on the living substance *per se* in some of its more minute structural aspects.

was not followed directly, but was seen later to have had a markedly degenerating course.

Wherever such abnormal processes of membrane formation by spinning were watched, the processes differed from the normal filaments in being more irregular and unrestrained in protoplasmic character ; there was less of direct thread formation, more of tufts or brush-like processes, which were unstable in progress and even returned altogether to the egg covering.

In the normal eggs the spinnings were swift, straight, smooth, direct, and continuous in their progress, neither ramifying nor returning. They seemed more homogeneous optically, and had not granules scattered along their course, as the abnormal spinnings often had for a considerable distance. Normally the threads came to be of about even length at about the same moment, while in abnormal eggs they were at a given moment unlike at almost all points of the periphery, the most perfect being formed always about the region of entrance of the first sperm.

In normal eggs, the threads, having reached a certain, but rather variable, distance from the egg, appeared to fuse at their tips and then to spread out their substance there, so as to form a ceiling film. Increase of outflowing substance soon thickened the pellicle thus made, and almost simultaneously the threads themselves proceeded to fuse along their length, either by access of material from the egg over them, their linear extension having been to a great degree stopped, or by a spreading out of their substance as they continued to be formed from the rear ; or perhaps even by exudations or secretions from them.

The distance from the egg at which the threads began to fuse at their tips was variable ; when it took place at but a short distance, the film so formed was raised and extended by continued elongation of the threads, the lengthwise *filling in* beginning then or later, as the case might be.

And in this manner was a striated membrane seen to be formed about sea-urchin eggs.

Starfish eggs. — It has been commonly observed that, just at the region where the so-called polar globules are expelled, the

cytoplasm shows amoeboid movements before, and for a short time after, these bodies are thrown off.

Under magnification of three thousand diameters, the lobe-like protrusions of the substance here were seen to be still further extended in filose processes, most like the outer portion of the *tuft* which receives the sperm, but finer and more thread-like, less protoplastically diffuse.

After the globules have been thrown off, these processes are for a short time withdrawn, while the general surface of the egg smooths itself out, restoring the general contour. For a short time only, however; for very soon the spinnings arise again from the pellicular surface, now in the form of still more delicate threads, or rays, which extend themselves towards the egg membrane, and even at moments attach themselves to this. They also surround the polar bodies with ramifications of extreme minuteness.

At other points of the periphery similar spinnings spring up, the egg being still in the single-celled stage, and thus is inaugurated a phenomenon which from this time persists with varying freedom until shortly before the closing of the opening into the cleavage cavity, across which the polar bodies lie. Then all peripheral spinnings cease, to burst out once more for a few moments all over the pellicle when the free-swimming life of the larva is begun, after which no more filose activities were seen from the exterior of the embryo.

The threads, or rays, formed from the pellicle in the one-celled stage are of extreme delicacy in the normal egg, and in many cases are but just perceptible with the powers named, except near their point of origin in the pellicle, where they are thicker and less viscid in appearance.

They branch sometimes near their base and sometimes further out from the egg, when in their more fluid states, for their viscosity and their refractive quality vary from moment to moment. Such branches take a somewhat wandering course often, and anastomose, or interlace, with the secondary, or even at times with the primary, processes near them, so as to form unstable networks. The branches are often as thick as the threads from which they arise.

The rays do not have always a radial direction as to the egg, not even at the moment of their formation, but may make with the surface of the cell almost any angle, even so acute an one as to be actually tangential.

They are moved slowly at times, as if from a hinge, or ball-and-socket joint, at their base and point of termination in the egg pellicle. They are seen also to bend sharply and suddenly at some point of their length, forming thus angles more or less approaching right angles. And at all points of their length they freely interchange varying states of viscosity; being here or there in the different filaments, or here or there at different points in the same filament, either in a state of fluid flux, or stiffly viscid to a point which may reach elastic rigidity.

The spinnings from the pellicle continue without intermission during all the internal preparations for caryokinesis, and for cell division, showing no constraint during aster formation, nor even in actual cleavage, except for a moment just before actual splitting of the surface begins, and just along the region, or coming path, of that splitting, for here they are more or less completely withdrawn.

As external cleavage begins, the rays nearest the actual splitting line show usually some agitated-looking bendings about, both from their base and along their course. Sometimes a quivering movement runs along them, with roughenings of the ray outlines exactly as is seen often in *Heliozoa*. At other times, in the earlier stages of the egg's history, such phenomena were seen from moment to moment in different rays at other regions.

The split of the first cleavage is now visible as a triangular cleft in the optical contour of the egg. In the moment this appears, there is a visible haste in the protoplasm of the rounded, divergent sides of the two cells in process of formation, to renew the spinning activities. The rounded optical edges may show slight amœboid modulation; and then, rapidly starting up, a number of processes extend themselves from each opposing surface, towards the other. This is quickly reached, for there is both haste and directness of

formation, and so the two halves become in fact re-united by their substance before they are well separated.

Following hard upon the physical splitting of the mass apart, similar spinnings from the newly separated surfaces spring up, so close indeed at the heels of the cleavage, that there is often scarcely the width of a half dozen such threads between those most newly formed and the still fused edges of the cleft. By the time the first cleavage is ended, and this does not take up many moments, the path of liquid between the sister cells is already crossed many times by minute and most delicate ray-like extensions, and strands, and even skeins of more tenuous threads.

During succeeding changes of contour, when the blastomeres round themselves up and then again flatten, and still again become slightly concave in the centre, yet oppose a more or less plane surface to each other, the spinnings never cease, although the processes are in a state of general instability.

Under a lower power, even one of 1500 diameters, the filose processes are wholly invisible, the surface of the cell presenting an optically unbroken surface, which to still lower powers looks smooth even.

While the cells lie opposite one another thus, there is some ramifying and divergent spinning among the threads, and anastomosis of some of the finer secondary filaments, but this is mostly near the central, slight hollowing out of the surfaces; those threads having their origin in the more viscous-looking, outer portion of this plane tending rather to form bands and strands of more refractive and direct character.

Later, when the cells begin to approach each other, the connecting filaments show a decided tendency to still more marked directness, and smoothness, and viscosity of texture. The lateral, branching spinning becomes rarer; and as the cells approach each other the band and rod-like connecting filaments become shorter and gain a look of *tenseness*; and flow between the two cells of protoplasm by way of these threads was not noticed.

The threads shorten, and as they shorten become thicker and more highly refractive, — that is, certain ones, for some of

the processes, notably those which spun latest, are now generally retracted.

The whole aspect of things is as if the blastomeres were being drawn together by contraction of some of the threads.

Moderate pressure upon the cover glass, slowly increased, tending to a mechanical forcing apart of the cells with more or less flattening, had a most curious effect, for it seemed rather to intensify and hasten the drawing together of the cells, and to increase the refractiveness of the threads, intensifying, rather than lessening, their optical characters.

The threads were not seen to be drawn out into finer and less refractive filaments by such pressure,—as would naturally be the case were their nature purely physical, like that of the threads left when Bütschli forced apart masses of his viscid oil foams,—but, as stated, if the pressure were slow and moderate, they held and even emphasized their peculiar character. In finally giving way, as sometimes happened when the pressure threatened to rupture the cell walls, they broke short off and then rounded their ends in a viscid, mucilaginous-looking manner before retraction, which soon took place.

Another curious fact was, that such mechanical pressure caused the egg to withdraw many peripheral processes, but seemed at the same time to increase, or re-stimulate, new formations from the opposing surfaces of cells. Later, the peripheral spinnings were renewed with even greater activity if the pressure were taken away; or, in cases where the cells were forced apart and the membrane remained intact, it being very plastic under slow pressure, the peripheral spinnings were not only renewed at points over the entire surface, but extended themselves through unaccustomed distances until they reached the other cells. These results were best gotten in the 8–16 cell stage, — which seemed peculiarly to favor them.

In the natural course of events in the typical, normal eggs, after the blastomeres were closely apposed to, or fused with, each other for a time, the same response to pressure was given by the cytoplasm of the new mass. *Pressure seemed, in short, to increase the physical resistance of the mass to crushing stress.* Since, if the cells so treated happened to be in that rhythm-

cally somewhat relaxed state between cleavages, they rounded themselves up under intermittent pressure instead of flattening; it is thought that the peripheral substance was instrumental in these changes.

After two sister cells are in close apposition to, and what may now, perhaps, be called safely, physiological union with, each other, there is offered to mechanical pressure an emphatic, and, if the pressure be slowly augmented, an increasing resistance. I found that shaking also increased the resistance to pressure. In making these pressure experiments I used a large, screw-adjustment compressorium, making marks upon the head of the screw and upon the supporting plate to estimate in a rough way the relative amounts of pressure. No doubt special devices for this purpose would bring to light an important series of differences.

After re-union of the first two blastomeres to form what may be termed a *dual mass*, matters progressed in the usual rhythmic way towards the second cleavage. During this time the spinning phenomena were persistent over the general periphery, the processes being longer and extending themselves to greater distances, which they may easily do because of increase in size of the egg membrane.

It may be difficult to convey a true idea of the extreme delicacy of these spinings from the egg pellicle. In attempting camera drawings, I found that even a fine needle point came much in one's way in efforts to follow their outlines. There were local thickenings and differences of aspect which could not be portrayed so at all, and the chief meaning of these lay in their very evanescence. The only way seemed to be, to receive as truthful and passive a brain photograph as possible, and to translate this into terms of permanent line and shape, getting with the camera a skeleton of relative lengths and general local character of the lines of living substance.

The rays showed all the characteristic differences known in Heliozoan rays; and when one says this, much is said of many variations. They were freely produced and as freely returned to the substance of the pellicle from which they arose.

All the while, the alveolar structure, which was clearly

traceable in the protoplasm underlying the pellicle, and much finer than that found in the more central portion of the mass, remained optically undisturbed by these displacements of a peripheral substance which formed indeed the so-called pellicular film of the structures of Bütschli.

In attaining a four-celled stage, the phenomena were practically a repetition of the first set described. After fusion, the blastomeres rounded themselves up and formed a hollow mass. The central cavity of this, which was the "cleavage cavity," of course, was filled with a network of interlaced and even anastomosed filaments. And still the periphery of the now quadruple mass spun as before.

The most active portion of the common periphery seemed to be always at the rounded sides of the partial cleft which marked the junction of each two, adhering blastomeres. The most freely ramifying and anastomosing processes seemed to come always from the inner surfaces which lined the cleavage cavity, and this portion of the general substance showed greater fluidity, which extended inwards some little distance in each cell.

During the rhythmic cell-flattenings, the very active portion of the periphery just mentioned was always most extended and flattened, so that under highest powers it showed a decided prominence. This, under lower powers, might possibly be seen as a slight modulation of the rounded surface-outline.

Here the filaments attaching themselves to the opposing surface would seem to pull it somewhat out of place, and here, as in the actual cleavage, there was a tendency to formation of fusing filaments, or strands and bands.

So matters continued throughout the cleavage up to the time of closure of the opening into the cleavage cavity, over, and sometimes within, which the polar bodies lay.

At all times the cleavage cavity was crossed by a variable number of threads, connecting distant cells as well as those nearer together; each cell seeming desirous of connection, though not persistently, with all other cells. The filaments were long, more or less direct, connections which in many cases ramified during their course, sending branches to several cells, or spinning by the way a network whose filaments

interlaced with, or joined themselves to, other filaments or networks met with.

The greatest number of spinnings seemed to be from the cells near the polar globules, and this region of the egg, it will be remembered, was the first to spin as well as the most active at all times. In this region was shown also most tendency among the peripheral filaments to ramification and anastomosis, especially when they crossed the path of certain spinnings from the polar bodies, shortly to be described; most capriciousness in their optical qualities, and interchange of fluid and viscid states, with greatest inclination to stiff viscosity.

At time of closure of the cleavage cavity pore, the cells about it flatten markedly, and at the same time become irregular in optical contour; the spinnings are then very strong and viscid-looking, and the protruding regions are seen to be attached by them to like regions of other cells. There follows much the same series of optical appearances among the filaments as when sister cells are drawn together, and for these reasons it seemed not unlikely that the filaments were actually instrumental in closure of the space between the cells.

After a closure of the space is effected, the general "ectoderm" cells, as they multiply, become gradually flattened exteriorly beneath their common pellicle, so that all seeming of intercellular clefts is obliterated. At the same time their inner ends protrude somewhat within the cavity, so that here there are marked intercellular clefts.

These clefts are crossed from cell to cell by delicate, hyaline, filose extensions of the cell pellicle; and from the ends of the cells, which are plainly more fluid than the outer portion, are produced other and much longer threads, which extend even across the whole cavity, binding the most distant cells into physiological and direct continuity.

Up to this time the external spinnings of the mass pellicle have been progressively somewhat less profuse. When the blastula has a sufficient number of cells for its free-swimming state these outer spinnings rather suddenly cease, but burst forth again over the surface of the pellicle as hair-like processes which quickly show irregular, contractile, or waving

motions. These become stronger, more rhythmic and organized in action, and soon form a sufficiently harmonious impulse to carry about the blastula as a free-swimming organism.

After invagination, those cells which as entoderm project, first as a plug and later as a tube, into the cleavage cavity, spun strongly and in a free protoplasmic manner from their portions facing the cavity, which, like the same region of the ectoderm cells, were more fluid in appearance. These entoderm spinings crossed the cavity to the ectoderm cells and in their course freely anastomosed in many places with the spinings of the latter.

After the mesenchyme cells were added to the group of internal cells, these, by spinning in even more free and profusely protoplasmic a manner, increased very greatly the number of intercellular connections.

It now became difficult to distinguish the various groups of spinings from each other; in the majority of cases where anastomosis had taken place, it was impossible, because new centres of spinning were formed of accumulated substance which might have come from anywhere. The network was also most unstable from moment to moment. Yet there were many tapering processes which could be connected with their point of origin.

Larval stages were followed up to quite late periods, even to time of formation of the proctodæum; and the internal spinings seemed at no time to pause or cease.

It should be pointed out that in the early stages of the segmenting egg the protoplasm which spins is not the least, but the most, highly organized portion of the cytoplasm. It is also the most viscous portion, as can readily be demonstrated by physical experiment.

Polar Globules.—Perhaps even more strange than these spinings from the egg were similar activities shown by the cytoplasmic envelope of the chromatin granules of the polar globules.

During that short period of quiescence for the egg pellicle, which was spoken of above as following the extrusion of the

polar bodies, thread-like processes with some wave-like change of contour were noted on these little lumps, the egg being at the time under magnification of three thousand diameters. These initial protoplasmic activities were extended, and multiplied from other portions of the hyaline protoplasm enveloping the nuclear substance.

Delicate threads and ray-like processes grew out on many sides, and the shape of the whole mass suffered change from moment to moment, both from the actual displacement thus caused and from an apparent pulling here or there of the little cells by their attached filaments and strands which had formed actual connection with the egg pellicle or membrane.

It seemed to me to be from this time, *i.e.*, when the threads from the polar bodies effected reunion with the parent cell, that the egg spinnings were again renewed. However this may be, it was certain that from this time forward, throughout the entire cleavage of the egg up to a late larval stage, the egg and these bodies were united by their individual spinnings, which formed an unstable, and at times evanescent, series of interlaced and anastomosed networks. These more or less surrounded the polar bodies and stretched across the cleavage pore.

The processes from the polar bodies were finer in general than the average egg filament, but this was variable, and along the finest filaments would often pass little masses, relatively large in quantity, of flowing protoplasm which might collect here, or there, and form nodes as it were, and these became centres of renewed and somewhat independent spinnings.

From moment to moment, the substance composing the network, or its islands of protoplasm, would return wholly to the polar bodies, to be again sent out in a new direction and to assume a new form.

The globules seemed to delight, especially later on in the egg history, in forming brush-like tufts, or skeins, of finest filaments, which often assumed a curious, superficial resemblance to a spindle, spreading at a little distance from their point of origin and then again drawing together at their point of fusion with some ray or strand, or with the nearest egg cell.

By shortening of the filaments, the position of the globules was changed, these being drawn here or there ; and later their shape underwent strange changes which forced one to correlate them with existing thread formations. Now they would be seen as irregularly spindle-shaped, with bundles of radiating filaments arising from each end of each body ; then as spherical masses, with Heliozoan-like rays extending from all sides nearly alike ; then again as colonial groupings of separated minor masses of protoplasm connected by filaments and having the chromatin granules distributed to some extent among the larger lumps ; then again, with filaments almost entirely withdrawn, as amoeboid shapes with delicate wave-like expansions of substance flowing in protean manner about the granules which were then perhaps collected together at some one point.

Delicate vacuolations of variable sizes appeared and disappeared in the main masses and in their smaller colonizing masses.

The protoplasmic granules were transported here and there along the processes, and the filaments underwent all those changes of optical quality which have been described in the egg spinnings.

The change of position and grouping of the chromatin granules were deeply interesting, for they were at times scattered, then drawn in line, then variously grouped. But I was unable to determine any coherent significance in these differences at the time, and dare not so much as guess that their arrangement was other than fortuitously and mechanically altered by flux of the surrounding substance.

The granules' presence, even singly, in any mass of the cytoplasmic spinnings certainly seemed to be correlated with more continuous, persistent, and in a way organized, displays of this sort.

From all the surrounding cells, passing filaments gave of their substance from moment to moment to the compound network which compassed the bodies about, and it was not always possible to know from what source a given portion of the network had come nor whither it was bound. It was always

possible to know some portions of the network as distinctively the polar bodies' own.

The position of the polar bodies, with relation to their distance from the egg membrane and the egg itself, varied also throughout the time of development up to this point.

Their substance, like that of the egg, frequently travelled to the membrane and there adhered, spinning backwards or anastomosing with such egg filaments as happened to be there.

At time of closure of the cleavage pore, the polar globules were shut inside the blastula, chiefly, it seemed, by action of the egg processes overpassing them and drawing together the cells above them; yet possibly they assisted also by some migratory movements of their own.

Inside the blastula, they could still be followed clearly for some time, until at last, after being involved in the web of spinings from the ectoderm cells and, in the gastrula, from the protoplasmic ends of entoderm cells, they were finally lost to sight in the still further additions given off from the mesenchyme cells. After this point their fate could no longer be followed, though it enlisted the strongest interest.

Towards the time of closure of the cleavage pore an increased tendency to viscosity and to organized action of the spinings was shown by the strange geometrical positions maintained by the lines of living substance.

In one instance where the conditions remained stable for some moments, a camera drawing was obtained of a certain arrangement which from a mechanical point of view was remarkable. One line of very viscid-seeming protoplasm formed an arc which would make part of a very large circle. The ends of this terminated in the angles formed by sharp bendings of two long rays having their origin in the two polar bodies then lying quite apart; and their termination in two cells of the egg which were not adjacent but separated by another cell. Thus it came about that one saw a viscous fluid in linear extension, maintaining the curve of a distinct arc, yet attached to the angles formed by what were, mechanically, four contending directions of tension. It might have been open to one to suppose that the angled lines were very viscid, and that the arc line was a less

viscid, hanging line of protoplasm, ~~had~~ not the existing conditions negated this view. The arc line was in fact far more dense and refractive, also thicker than the angled lines between which it held its place ; and it did not hang.

Appearances were far more in favor of its being this line which bent the longer lines into their angles. Yet in such a state of tension, how explain the curve? I have dwelt at length upon this single phenomenon because it is typical of the difficulties which everywhere beset physical and mechanical interpretations when one brings them into contact with the facts of the living substance.

The hyaline substance of the polar bodies frequently flowed along the filaments of the egg, and gathering together at some point spun characteristic brush and skein formations, or made nodes for diverse sorts of protoplasmic phenomena.

It was a noteworthy feature of the polar spinnings that they so frequently grouped themselves in spindle-shaped bundles, and showed a marked vesicular structure in their substance at most times except during certain changes of viscosity.

Concentric chains and lines of vesicles gave often by their arrangement the aspect of a spindle to the whole polar body, which the outline of the mass at the same time emphasized. In the brush and spindle-like spinning products there was often a very distinct chain-like arrangement of vesicles forming the threads. These were of course the larger and more stable processes.

Description of the filose phenomena of these eggs is an almost inexhaustible subject, and I give only the more patent phenomena.

The activities of the polar bodies did not decrease as time passed after their extrusion from the egg, but rather increased, both as to amount and as to the controlled and ordered nature of the phenomena.

In other words, instead of their losing at all their individuality and becoming more like non-living and excreted substances, they rather gained than lost in independence of action and the vigor and vitality of their organized activities.

Whether this were due to some influence exerted upon them by the intimate connection of the egg spinnings with them,

through which physiological relation they might possibly share in the growing individuality of the major body, it is impossible to say. I am inclined to think that although they undoubtedly show strange freedom and initiative energy, although they seem to possess all that is requisite for what would be termed from a Protozoan standpoint, a complete organism ; yet, since they are also an integral part of the egg substance and of the complex system of egg phenomena, they must share largely in the sympathetic interactions and coöperative physical and physiological phrasings of the powers of the whole material, as well as in a progressive subjugation of the whole life-machine to an ever-increasing despotism of parts.

In the *Echinus* and *Arbacia* eggs also the spinnings were seen between cells, although with far less freedom than in *Asterias*. The sea-urchin threads were fewer, in comparison, more direct, and less given to forming networks and side-spinnings. They arose from a more distinct, and thicker, hyaline covering of the egg, which seemed structureless under the highest powers, yet the spinnings themselves showed often a vesicular tendency and structure, albeit of the finest. Over the sea-urchin filaments granules as well as little lumps of protoplasm streamed at times. In the four-celled stage, the small cleavage cavity was crossed by a number of threads between the cells, and thereafter whenever, and for as long as, interspaces between the cells could be seen, spinnings crossed these and bound the associated cells into an intimate union.

Here, as in starfish eggs, the most active and sensitive portion in each cell was where it began to curve away from another cell.

In both starfish and *Echinus* eggs, after any artificial separation of the cells by such pressure as did not rupture the egg membrane, and even in some cases where this actually occurred, the spinnings were increased in number and the length of the threads was extended until connection was again established, as if indeed they sought for their lost comrades.

To the very natural question whether these filose activities of the eggs were not indeed abnormal phenomena, it is to

be answered that the same question having great weight in the observer's mind, every effort was made to learn the truth.

Thus much was made sure. The eggs can be stimulated by additional heat, by polyspermy, by adverse states of the water such as are caused by too long confinement, by mechanical pressure, or by being fertilized when in a still immature condition, to spin far more freely.

But the protoplasmic phenomena in these cases are distinctly different from those of what I was impelled to believe were normal eggs.

The character of the formations, the quantity of the egg substance, and the use made of it during the normal and abnormal states, are so different, so characteristic, that, after following a dozen or two specimens of abnormal spinnings in all grades of pathology, one feels an almost unshakable assurance in the nature of the true and normal process, and would almost be willing to diagnose the state of an egg by the very character of its spinnings in any given case.

Immature eggs being fertilized, or over-fertilized, give themselves up to uncontrolled spinning activities which in many cases end by the whole mass being drawn out of form and position and distributed through the surrounding space in a granular and unevenly vesiculate, or a partially structureless, network of substance. The transported substance may mass itself here or there, adhering perhaps to the membrane and thence setting up from broken, or partly isolated, lumps and nodes of protoplasm new centres of activity.

Traversing relatively large spaces, often by way of invisible extensions of substance, the deported protoplasm accumulates at this or that point; and then may disappear, to come again within optical reach as a new current from nowhere flowing into or onto some filament in plain sight; or as small lumps of substance, growing from some invisible source, suspended in an otherwise empty space, their true source being some distant filament, or the egg mass from which outflowing currents take their way, and pass at some point from one's power to trace them optically.

Mature and properly fertilized eggs when heated too much or kept too closely confined, cease their normal, delicate spinning to burst into profuse transpositions of their substance, which may cause even considerable distortion of their form, or, according to the degree of abnormality produced, may be visible only as excess of the usual processes. If not too much affected, such slight abnormalities may be cured and the perfect larva form as usual.

If the adverse conditions are continued, such eggs often cease from all further cell division and exhaust their powers in perfect orgies of spinnings, as just described for abnormal eggs.

Protoplasm artificially pressed from normal starfish eggs in early stages spins characteristically like abnormal unruptured eggs and cells, not like those in normal condition. Such activities varied also their quantity and character in correlation with certain rhythms of difference of physical quality which marked the living substance during development of both starfish and *Echinus* eggs. These rhythms are treated of in the forthcoming essay mentioned above.

There was every optical evidence of actual interchange of substance by the cells of developing starfish eggs and also *Echinus* eggs by way of the filose processes; for a procession of granules along a filament for some moments in one direction, sometimes ended by abrupt withdrawal of the filament. Whether these granules returned later along some other filament could not of course be determined; but even so, they would have been for a time inhabitants of the cell in which they were left, and so also would the substance of whose passing they were clearest indications.

A most noteworthy thing was made plain by this series of observations. The vesicular structure of Bütschli, and that modification of it termed by him the "alveolar layer," clearly traceable under favorable optical conditions in these eggs, exists and remains optically undisturbed for moments at a time during such peripheral activities as can drain the egg of considerable amounts of its substance, yet pass undetected under the powers which have been commonly used for observing developmental phenomena.

Further, there is present and visible under these lower powers, and capable of "preservation," a perfect, pellicle-like covering of ectosarc-like substance which under quite high powers may show only such surface roughening as would pass as finely granular, or seem smooth even, and yet be extending itself all the while in hundreds of processes which are most powerful determining factors in the scheme of development.

In the case of the polar bodies as in the cell division, it becomes plain that the separation or isolation of portions of its substance by the developing egg is but an optical illusion; and that the space separation between sister cells, which has been taken to be actual and complete and unbridged by living substance, and that cavity or space between all the cells of the blastula known as the cleavage cavity; are deceptive in the highest degree, being in fact bridged by all the cells concerned by means of extensions of their living substance.

At all times the cleavage of the mass of these eggs into portions which simulate units, is seen to be but a mask for actual continuity of the substance of the whole throughout all its subdivisions in cell form.

More than this, the cell substance is seen to have some sort of deliberateness, or purpose, in its spinning activities, for where the space between blastomeres is artificially increased far beyond the accustomed limit, it hastens to cross by unaccustomed degrees of extension, not resting till it has reached the missing masses and reestablished union with them.

A reasonable summing up of the phenomena described,—weighted by a host of subtle, modifying and restraining evidences which in such delicate phenomena must always exist over and above the description,—would seem to be as follows.

The facts point to a physiological drawing together of the cells, rather than to any physical and chemical "cyto-tropismus":

To a physiological, rather than a physical reaction to mechanical stimulus of pressure or shaking :

To a physiological, rather than a physico-chemical, cause of the spinning activities :

To actual and physiological communion as well as physical connection between cells after and during their formation :

To some interchange of the protoplasm between cells, which may be but temporary, or may be part of the organising phenomena :

To an actual desire on the part of the cells for such physical connection and physiological communion among themselves; and a coherent attempt to regain and continue these conditions after they have been artificially destroyed :

To a continued, and to great degree independent, existence of the polar bodies after their extrusion: and to their acting as part of the coalition of cells up to a late period, yet without self-multiplication, and with marked distinctive features of their own, which are peculiarly protoplasmic.

The facts assert also:

That the peripheral substance of egg and cells is freely protoplasmic, despite its appearance under less magnification of being a smooth and stable pellicle :

That the ectosarcial formations of these Metazoan eggs are possessed of tactile power such as characterizes similar modifications of the substance in protoplasts :

That considerable deportation of living substance can take place from egg or cells in a manner invisible to even quite high powers (as 1500 diameters), and yet to an extent producing a measurable diminution in size of the mass from which it is carried :

That, in short, the measure of the living facts cannot be taken truly with anything less than our best optical resources, and that even these fail of perfect adequacy :

That apart from the grosser organization of the egg, which we have had spread out to some extent before us by reagents, there exists a minuter mechanism which these fail to record, or even hint to us; and that beneath the interactions of those grosser masses which seem so complete as seen under lower powers, there are supplementary and strongly causative interactions of minuter portions of the substance composing them, which are in part visible with our highest powers and which are from some points of view curiously contradictory in seeming :

That while the grosser organization acts through more definite and stable structures, the finer, and heretofore unseen,

organization acts by unstable and freely protoplasmic phenomena and structures :

That while the phenomena pertaining to the organism as composed of these grosser masses and structures we call cells, are of vast importance ; the phenomena pertaining to the organism as composed of those minuter and unstable portions which go to make up the cells, and which taken together throughout the whole mass, as well as separately, go to make up the protoplasm *per se*, — *the living substance, as such* ; — are of transcending importance, since they prove to be control phenomena for the former. In saying this I have in mind the familiar phenomena of caryokinesis, and certain unfamiliar phenomena of re-arrangements of the cytoplasmic substance which are treated of in the forthcoming paper, and which undoubtedly strengthen the expression here of these conclusions.

Here I will limit myself to saying that whatever may be the significance of the cell wall in the development of these eggs, it surely cannot be thought a separator, in either a physical or physiological sense, of the cell contents from other portions of the common mass.

If we look upon the cell wall as a part of the machinery of the embryo, the larva, the coming or immediately present organism, that is, as an organ of the mass of living substance, just as is the nuclear membrane, or any other local modification for physiological purposes, we shall probably be nearer the truth than if we keep to an earlier conception, and hold that, for the living substance, cell walls a prison make.

BALTIMORE, October, 1896.

1.

THE ORIGIN OF THE EGG CENTROSOMES.

A. D. MEAD.

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THE observations recorded in this paper were made upon the eggs of the marine annelid *Chaetopterus pergamentaceus*, procured at the Marine Biological Laboratory at Woods Holl, Mass., where I was enabled to work during the past summer through the courtesy of its Director.

My best preparations were obtained by fixing the eggs with picro-acetic acid, and staining with Heidenhain's iron-alum hæmatoxylin, followed by orange G. The slides were left in the 4% iron-alum for half an hour, rinsed, and left in $\frac{1}{2}\%$ hæmatoxylin for twelve hours. After drawing the color with iron-alum, the slides were dipped in the aqueous solution of orange G. Hermann's fluid, Flemming's fluid (weaker), and a mixture of Hermann and formalin also gave satisfactory results, though the staining was not so brilliant. Sublimate acetic usually works havoc in the region of the astrosphere. In a previous paper¹ I stated that "until the entrance of the spermatozoön, the egg remains with the first maturation spindle in the equatorial-plate stage." The earlier stages were not seen at that time.

During the past summer, however, on taking the precaution to preserve the ovaries, together with the loose eggs, immediately after dissecting them out into sea-water, I was able to obtain a complete series of stages previous to the formation of the first maturation spindle.

The cytoplasm of the smallest ovarian eggs is compact, and this gives to the eggs when stained an almost uniform dark purple color. The increase in size is due in great measure to the accumulation of yolk, the distribution of which is accompanied by noteworthy changes in the appearance of the cytoplasm. The yolk is laid down in the form of yellow-staining

¹ Some observations on the maturation and fecundation of *Chaetopterus pergamentaceus*, Cuvier. *Journ. of Morph.*, vol. X, No. 1.

granules, which are more numerous at first near the periphery of the egg. The granules are held in the meshes of a reticulum formed of beaded strands of cytoplasm, whose purple color is in striking contrast to the yellow yolk.

In most ovarian stages only a part of the cytoplasm presents the loose reticular appearance; the rest remains as dark purple masses. Occasionally sections show but one of these masses, crescentic in outline, located near the nucleus. I presume that this is equivalent to the "paranucleus" or "yolk-nucleus" of certain authors. These masses are not homogeneous, but resolve themselves into a radially compressed cytoplasmic network,

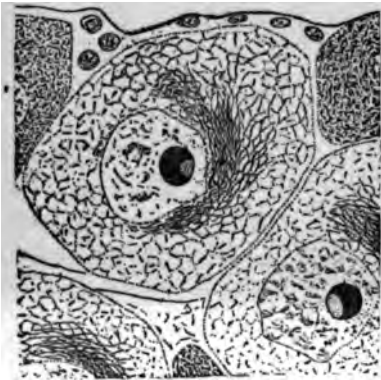


FIG. I. — Section of ovary. Camera. Dissolution of the paranucleus.

the strands of which are often clearly visible and are continuous with the more open network which contains the yolk. At first they fill the larger part of the egg outside the nucleus; but as the egg accumulates yolk the meshes at the periphery of the masses expand into an open reticulum so as to occupy the increasing dimensions of the egg (Fig. I).

Through this process of continuous fraying out, the masses

become thinner and thinner and are finally completely resolved into the cytoreticulum.

The reticulum can be traced with ease to the extreme periphery of the egg, where in section one can follow a continuous beaded line entirely around the egg. The nuclear membrane is evidently a part of the same network. The general appearance of the reticulum varies with the age, the older ovarian eggs having the nodes the most pronounced. In eggs freed or about to be freed from the surface of the ovary, the nodes become less frequent and still more prominent, until at length a large portion of the reticulum is transformed into a multitude of small asters (Fig. II). Many of them, however, are by no means diminutive, particularly those in the vicinity of the

nucleus. All gradations in size occur, but at a certain stage many of the larger ones are approximately equal both in size and distinctness. Frequently one can count from fifteen to twenty very distinct asters in a single section. These structures correspond closely to "secondary mechanical centers" of Reinke,¹ and I will call them the *secondary asters*. While yet distinct from one another, they are often so near together that their rays intercross.

It is not long before two of the asters become predominant, — *primary asters*. Their rays increase in number and length,

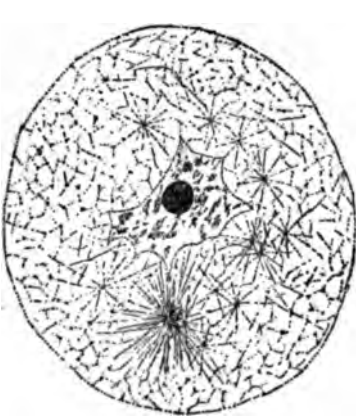


FIG. II. — Section of egg free from ovary, showing secondary asters. Camera.



FIG. III. — Section of egg in a stage later than Fig. II. Only the two primary centers are present. Camera.

apparently at the cost of the secondary asters, for the latter gradually evanesce with the further development of the former, and at length the cytoplasm possesses only two well-marked centers of radiation (Fig. III).² The primary asters lie near the germinal vesicle, usually about 90° apart, but their relative position is subject to considerable variation in different eggs. *They are destined to be the asters of the first maturation spindle.* Each of them has for its center a perfectly definite deeply stained centrosome (centriole), surrounded now by a lighter area from which the rays diverge. The nuclear membrane

¹ Reinke: Zellstudien II. *Arch. f. mikr. Anat.*, Bd. XI., p. 276. Peritoneal cells of the larval salamander.

² The initial predominance and further growth of the primary asters certainly appears to be due in part to the actual coalescence of smaller asters.

invaginates in the vicinity of each aster in the manner already described by several authors.¹ It is afterwards resolved into the cytoreticulum, though the huge nucleolus and other features of the nucleus do not immediately disappear.

Definite chromosomes are seen to be attached to the achromatic fibrils of the two asters, and to take their position in the equatorial plate in the ordinary way. The centrosomes become doubled and are demonstrable in each aster as two exquisitely clear dark dots in the midst of the light yellow centrosphere. The spindle thus formed swings around to occupy a radial position at the periphery of the egg, and remains in the metaphase until the sperm enters. On the entrance of the sperm the karyokinetic activity is resumed and the maturation processes are completed.² Usually, before fertilization, the nucleolus of the germinal vesicle breaks up and disappears; the last traces of it are seen in the neighborhood of the maturation spindle.

The foregoing observations convince me that the asters and centrosomes in the *Chaetopterus* ovum arise by a modification of the cytoplasmic reticulum. The phenomena of their origin and their relation to the secondary asters are similar to those described by Reinke³ in the tissue cells of the larval salamander.

Watasé says he has "seen in the egg of *Macrobodella* a series of thirteen asters, ranging from a diminutive aster with a microsome for its center to the normal aster with a veritable centrosome."⁴

The fact that in *Chaetopterus* the secondary and primary asters were formed when the eggs were transferred from the body-cavity of the worm into the sea-water, suggests a comparison of these phenomena with the production of "artificial centrospheres" in sea-urchin eggs by adding more salt.⁵

¹ Compare Wheeler: *Myzostoma*. *Journ. of Morph.*, vol. X, No. 1. Griffin: *Thalassema*. *Trans. N. Y. Acad. Sci.*, June 2, 1896.

² Mead: *loc. cit.*

³ Reinke: *loc. cit.*

⁴ Watasé: *Origin of the Centrosome*. *Biol. Lectures, Marine Biological Laboratory*, 1894, p. 285.

⁵ Morgan: *The Production of Artificial Centrospheres*. *Archiv f. Entwicklungsmechanik*, Bd. III, Heft 3.

DEVELOPMENT OF THE HUMAN COELOM.

FRANKLIN P. MALL.

Four years ago I wrote a general article on the coelom for Wood's *Handbook of Medical Science*, in which was emphasized the separation of the body cavity from the extraembryonic coelom. Since then I have had opportunity to extend my observation to the human embryo, and therefore make this communication.

Unfortunately, there are no data regarding the beginning of the coelom in the human embryo, and in all probability none will ever be found. The smallest human ovum ever seen is that described by Reichert.¹ It was obtained from a woman who had committed suicide, on account of pregnancy, forty-one days after the beginning of the last menstrual period. It was therefore presumably about thirteen days old. This ovum, which is pictured in every text-book, was 5.5×3.3 mm. in diameter, was surrounded by a zone of villi leaving two poles bare, and contained in its interior a mass of cells measuring 1.5×1.75 mm. All the space between this inner mass and the chorion is the coelom, and regarding its origin, we can no more than speculate.

During the last few years three other human ova, slightly larger than Reichert's, have been cut into sections, thus permitting a more careful study of their contents.² The dimensions and approximate ages of these embryos are given in the table on the following page.

It is noticeable that in the three embryos just mentioned, as well as in the remaining four of the table, the size of the whole egg does not correspond with the size of the embryo, nor with its age. I do not think that this great variation in the size of the chorionic vesicle is altogether due to the method of harden-

¹ Reichert: Abhandl. d. kgl. Akad. d. Wiss., Berlin, 1873.

² Von Spee: His's Archiv, 1889; Mall: Anatom. Anzeiger, 1893; Johns Hopkins Hospital Bulletin, 1893; and von Spee: His's Archiv, 1896.

TABLE OF YOUNG HUMAN OVA.

OBSERVER.	DIAMETER OF EMBRYONIC VESICLE.	DIAMETER OF OVUM.	TIME BETWEEN FIRST LAPSED PERIOD AND ABORTION.
Mall (No. XI) . . .	1.5 × 1 mm.	10. × 7. mm.	13 days
Reichert	1.75 × 1.5 "	5.5 × 3.3 "	14 "
Von Spee (v. H.) . .	1.84 × 1.083 "	6. × 4.5 "	12* "
Von Spee (Gle.) . . .	2. × 2. "	10. × 8.5 "	12* "
Mall (No. XVI) . . .	2.1 × 2.1 "	18. × 8. "	13 "
His (Lg.)	2.15 × 2. "	15. × 12.5 "	12 "
Von Spee	2.69 × 3. "	15. × 14. "	14 "
Janošik	3. × 4. "	8. "	15 "

* These are all of the authentic young human ova I can collect from the literature giving all of their measurements as well as the menstrual history of the mother. In both of von Spee's cases the time between the abortion and the end of the last period is given; in embryo v. H. the time is given as "exactly five weeks," while in embryo Gle. "five weeks" is given. If we estimate the duration of menstruation as five days and its frequency twenty-eight days, then the time between the first lapsed period and the abortion is twelve days, as I have given it in the table.

ing the specimen. Just at this time the growth of the chorion is precocious, as is also the case in the dog,¹ rabbit,² and monkey.³

The papers by Bischoff and by Selenka are worthy of the most careful study by every embryologist, and I take the liberty of rearranging some of Bischoff's data on the development of the dog. His observations are very extensive, and give us the basis for our present ideas of the passage of the ovum into the uterine tube after fertilization. Unfortunately, they were made before the time of sectioning specimens, yet they are more complete than most researches relating to this subject published since his time.

The portion I tabulate relates to the size of the embryonic mass or vesicle, the size of the ovum, and its approximate age. As far as I have been able to determine, these data taken from the dog are still the most important ones with which we can

¹ Bischoff: *Entwicklungsgeschichte des Hundes Eies*, Braunschweig, 1845.

² Bischoff: *Entwicklungsgeschichte des Kaninchen Eies*, Braunschweig, 1842.

³ Selenka: *Studien über Entwicklungsgeschichte des Thiers*, Heft 5, Wiesbaden, 1892.

compare the human ovum. Embryologists are accustomed to state that the age of a human ovum is to be reckoned from the beginning of the first lapsed period, and I think that Bischoff's observation upon the size and growth of the dog's ovum corroborates this view. He found that the ova left the ovary during the rutting period, but the exact date could never be determined. Neither did the time of copulation determine the ovulation. As a rule, it took twenty-four hours or less after copulation for the spermatozoa to reach the ovary, and about the same time is required for the ovum to reach the beginning of the uterine tube after ovulation. So if ovulation and copulation took place at the same time, fertilization of the ovum could not take place until twenty-four hours later.

In Bischoff's tables he often rates the age of an ovum from the first or from the last copulation, or from the beginning or from the end of the rutting period. I have attempted to tabulate his specimens from all four of these dates, but in none of the attempts did the size of the ova correspond with their respective dates. Often eggs of a given date were smaller and developed to a less degree than ova presumably younger. After much difficulty I finally constructed a table in which the size of the ovum and its age correspond. A number of the ova published by Bischoff were obtained from the same animal by removing half of the uterus at one time and the remaining half the next day. In each portion a number of ova were found, and they were usually of about the same stage of development. By this method of procedure it is possible to determine very accurately the growth of the ovum from one stage to one twenty-four hours later. So, by gradually plodding through the specimens published by Bischoff, it was possible for me to correct his data completely. It is remarkable, as the table shows, how slowly the development takes place in the early stages, and about ten days are required before the ovum is one millimeter in diameter. On the fifteenth or sixteenth day the ovum is about as large as the human ovum described by Reichert (see table).

Similar results can also be obtained from the various papers published on the rabbit's embryo. Its development, however,

is considerably more rapid than the dog's, as the period of gestation is but thirty days.

Recently Selenka has given some of his results relating to the development of the monkey. The most valuable specimen relating to the early development of higher animals was unfor-

TABLE OF AGE AND SIZE OF THE DOG'S OVUM.
(COMPILED FROM BISCHOFF.)

AGE.	DIAMETER OF OVUM.	DIAMETER OF EMBRYONIC MASS.	STAGE.
1 day.	.15 mm.		1 cell.
2 days.	.14 "		2 cells.
3 "	.14 "		4 "
4 "	.16 "		12 "
5 "	.16 "		64 "
6 "	.18 "		Mulberry.
7 "	.20 "		"
8 "	.21 "		"
9 "	.28 "		"
10 "	.30 "	.07 mm.	
11 "	1. "	.16 "	
12 "	2. "	.24 "	
13 "	3. "	.43 "	
14 "	4. "	.5 "	
15 "	5. "	1. "	
16 "	5. "	2. "	
16½ "	6. "	3. "	

It has been somewhat difficult to compile this table, as Bischoff's measurements are all given in Paris lines. My measurements are taken in great part from his figures, and I think that these are very accurate.

tunately lost, but its age and dimensions are preserved for us, and are of value in the determination of the age of human ova. The ovum came from a monkey kept in confinement which was killed eight days after copulation. If we estimate one or two days required before fertilization, this ovum cannot be over seven days old. This suggests that the early stage of this variety of monkey is developed more rapidly than that of the dog.

DEVELOPMENT OF THE MONKEY. (FROM SELENKA.)

	DIAMETER OF OVUM.	DIAMETER OF EMBRYONIC VESICLE.
<i>Semnopithecus maurus</i>	1.5 mm.	.3 mm. ¹
<i>Semnopithecus pruinosus</i>	6. "	.5 "
<i>Cercocebus cynomolgus</i>	5. "	.5 "
<i>Cercocebus cynomolgus</i>	10. "	2.4 " ²

The pictures Selenka gives indicate that the development of a monkey's ovum is identical with that of the human ovum. At any rate, the few specimens Selenka publishes give results which are equal to the great number of specimens of human ova which have been published. This only indicates that many of the interesting problems relating to early human development will probably be solved by the study of the monkey's ovum. There is but little doubt now that young monkeys' ova will soon be obtained for study.

MATERIAL EMPLOYED.

During the last few years I have obtained a number of young human embryos from physicians in different portions of the United States, and to them I am under all obligation for the present study as well as for some others which are to follow. Nearly all of the specimens which I give in a table are well preserved, and a number of them are preserved excellently. All of the specimens were stained in alum carmine, and with the exception of Nos. XVII, XLIII, and LVII were cut transversely. These three were cut in sagittal sections.

All of the specimens were hardened in alcohol, the value of which method I have repeatedly emphasized to my friends, and do continue to emphasize to those who may preserve specimens for my use in the future.³

¹ Not an embryonic vesicle, but only a disc.

² Neurenteric canal present.

³ Embryologists usually recommend that human embryos should be hardened by placing them in dilute alcohol and then gradually increasing the strength of the alcohol. It has been my experience that by this treatment the specimen is injured by maceration due to the weak alcohol. A few years ago I emphasized the fact that the whole ovum should be placed in a large quantity of strong alcohol as soon as possible. It should be handled as little as possible before hardening it, thus preventing mechanical injury. By leaving the ovum closed the alcohol must first penetrate the chorionic and amniotic fluids before it reaches the embryo, and thus,

Nearly all of the embryos were drawn or photographed to scale and then carefully cut into sections from ten μ to fifty μ thick. I find that for purposes of reconstruction it is a mistake to cut the sections very thin. Yet in small specimens, as Nos. XI and XII, the specimens were cut thin to permit of careful

LIST OF EMBRYOS STUDIED.

No.	LENGTH IN MILLIMETERS.		FROM WHOM OBTAINED.
	V. B. ¹	N. B.	
XI	—	—	Dr. Kittredge, Nashua, N. H.
XII	2.1	—	Dr. Ellis, Elkton, Md.
III	2.2	—	Prof. His, Leipzig, Germany.
XIX	5.5	4.5	Dr. Williams, Baltimore, Md.
XVIII	7.	7.	Dr. Douglas, Nashville, Tenn.
II	3.	7.	Dr. C. O. Miller, Baltimore, Md.
IV	—	7.	Dr. Williams, Baltimore, Md.
XLIII	15.	13.	Dr. Hooker, Baltimore, Md.
VIII	17.	14.	Dr. Ritter, Brooklyn, N. Y.
IX	17.	14.	Dr. Eycleshymer, Chicago, Ill.
V	18.5	17.	Dr. Kittredge, Nashua, N. H.
XLII	18.	15.	Dr. Wills, Los Angeles, Cal.
XXVII	18.	16.	Dr. Cottrell, Louisville, Ky.
XXVIII	19.	18.	Dr. Sewall, Denver, Col.
VII	19.5	18.	Dr. Booker, Baltimore, Md.
XXII	20.	18.	Dr. Snively, Waynesboro, Penn.
LVII	23.	20.	Dr. Howard, Cleveland, Ohio.
VI	24.	—	Dr. C. O. Miller, Baltimore, Md.
X	24.	20.	Dr. W. S. Miller, Madison, Wis.
XLV	28.	19.	Dr. Douglas, Nashville, Tenn.
XXXIV	80.	60.	Dr. Ellis, Elkton, Md.
XLVIII	130.	110.	Dr. Wilson, Worcester, Mass.

without placing the embryo first in weak alcohol, it naturally passes through the successive dilutions of alcohol before it is completely hardened.

It is very injurious to these delicate specimens to be wrapped in cotton before they are sent by mail or express. A perfect method is to place the preserved specimen in a bottle filled completely with alcohol, thus imitating the condition of a *fœtus in utero*. If there is no air or cotton in the bottle containing the embryo it is almost impossible to injure the embryo by shaking it.

Since I have emphasized this method of preservation (Johns Hopkins Hospital Bulletin, 1893), I have obtained a number of specimens excellent in every respect. These specimens are not distorted, nor macerated, nor shrunken.

¹ V. B. and N. B. indicate the length of the embryo measured from the vertex to the breech and from the nape of the neck to the breech, respectively.

cytogenic studies also. In most of the specimens photographs or an additional series of sections were made of the chorion and amnion in order to study the variation of these structures.

Embryos XI,¹ XII, and II² were completely reconstructed in wax by the method of Born. Nos. IX, VI, and X were reconstructed in part by Born's method and finished by His's method of reconstruction. The abdominal viscera of Nos. VI, IX, X, XXXIV, XLV, and XLVIII were modeled by Born's method.

The mechanical portion of reconstruction has been simplified to a great extent by a special apparatus used in the Anatomical Laboratory,³ which enables us to employ a modeler. The sections are projected upon a screen, to which the wax plate is attached. By working in a dark room with this apparatus it is easy to direct a modeler to draw the outlines accurately. He can then cut them out, and all that remains to be done is to pile the pieces and then blend them.

THE COELOM IN YOUNG OVA.

All of the young human ova which have been described contain within them a cavity, lined with mesoderm; this is the coelom, bounded by the somatopleure on the outside and by the splanchnopleure on the inside. This arrangement, as shown by a number of diagrams by recent authors, is very unlike the appearance of the blastodermic membranes of many of the lower mammals, and it is necessary therefore that we should revise our conception of the formation of the amnion in the human embryo.

The ova recently published by Graf Spee indicate that the amnion must be formed very early, and, since it is completed before the medullary grooves begin, we must admit now that it is formed much the same as it is in many rodents, *i.e.*, by apparent inversion of the membrane. When Bischoff⁴ first described inversion of the membrane in guinea pigs it seemed

¹ Mall: *Anatom. Anz.*

² Mall: *Journ. of Morph.*, vol. V.

³ Hoen: *Johns Hopkins Hospital Bulletin*, 1896.

⁴ Bischoff: *Entwickl. d. Meerschweinschens*, Giessen, 1852.

like a paradox, but, since the comparative methods of study have been introduced, inversion only means that the amnion is completed before the medullary groove begins to form. This alteration of the development of the amnion and the medullary groove makes the body of the embryo develop on a concave surface instead of on a convex one, thus apparently making the embryo inverted, as is the case in the guinea pig.

Closely associated with inversion of the blastodermic membrane is the formation of an additional layer of cells, discovered by Rauber,¹ the importance of which has been emphasized by Selenka and others. Rauber's layer is so marked in the rabbit that it was at first believed to be the true ectoderm. The fate of Rauber's layer has not been sufficiently studied to interpret it completely, and our ideas regarding it will not improbably require some revision. In many rodents Rauber's layer becomes markedly thickened on one side of the ovum, forming a support, or *Träger*, for the ovum. The relation of Rauber's layer to the *Träger* is shown beautifully by Selenka² on Plate XVI of his monograph.

The question which interests us here is whether the inversion of the blastodermic membrane as well as the discovery of Rauber's layer aids us in advancing a theory of the development of the germ layers of the human embryo, and thus in turn to explain the large coelom as found in all of the earliest human ova. I realize fully that any such effort will not be final, yet I believe that it will aid us to understand better the relation of the membranes as found in the human ovum.

In looking over the illustrations of the development of animals closely related to man, one is struck with the similarity of the arrangement of the membranes to those described for the human ovum by Graf Spee. One must compare only plates XXXV-XXXVIII of Selenka's³ paper with the two plates accompanying Graf Spee's⁴ to be convinced that the early development of monkeys is almost identical with that of man. Yet Selenka's researches on monkeys do not help us a great

¹ Rauber: Sitzungsber. d. Naturforcher Gesellsch., Leipzig, 1875.

² Selenka: Studien über Entwickl. d. Thiere, Heft 3, 1884.

³ Selenka: Studien, etc., Heft 5, Erste u. Zweite Hälfte, 1891, 1892.

⁴ His's Archiv, 1889 and 1896.

deal; they only show us that the monkey's development is like that of man. In monkeys we have also the precocious chorion and the early amnion and the large coelom between the umbilical vesicle and the chorion. The marked difference is that the amnion is attached to the chorion along its dorsal side, while in the human embryo this is only the case along the posterior end of the amnion. The attachment of the amnion along the chorion suggests that the embryonic plate separated from the exterior of the ovum along this point, as Selenka thinks he observed in a very young ovum only 1.5 mm. in diameter. Unfortunately, the most valuable specimen was injured in its preparation,¹ and Selenka did not trust himself to give any illustrations of it.

With the amnion attached at its dorsal end to the chorion, we understand why the entodermal end of the allantois must grow around an angle to reach the chorion (Selenka, Plate XXXVII, Fig. 5). Somewhat the same arrangement has been described by Graf Spee² in his embryo Gle., but the curve is by no means as marked, indicating that the attachment of the embryo to the chorion is along its posterior end, as shown by His³ in his well-known diagram of the formation of the amnion.

Regarding the very early stages of monkeys and man it is better that we make comparisons with animals most nearly related to them, and now we have careful studies of the very early stages of Chiroptera at our disposal. I believe that Selenka's⁴ study of the development of *Pteropus edulis* gives us the key for the comparison of the formation of the blastodermic membranes in mammals. Recent investigations by Duval⁵ on different families of Chiroptera appear to confirm the work of Selenka on *Pteropus*.

In order to illustrate these points more clearly I have made diagrams of three of Selenka's figures of *Pteropus*. Fig. 1 is from an ovum covered completely with two layers of cells,

¹ Selenka : Studien, 1891, p. 201.

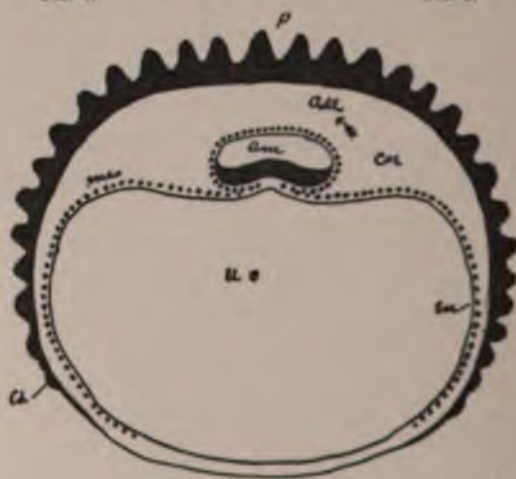
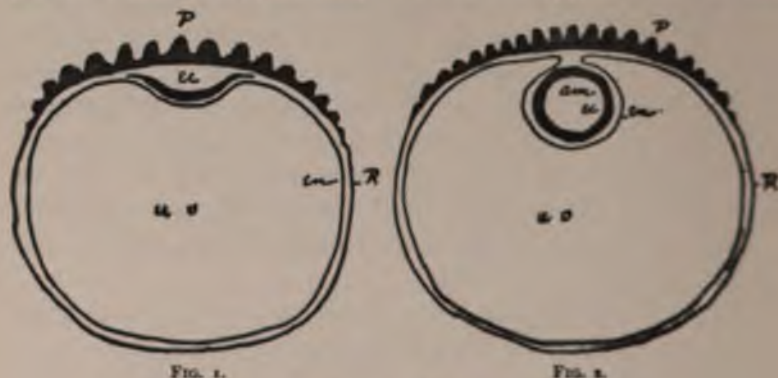
² Graf Spee : His's Archiv, 1896, Taf. I, Fig. 1.

³ His: Anat. mensch. Embryonen, Theil I, p. 171.

⁴ Selenka : Studien, 1892, p. 209.

⁵ Duval: Jour. de l'Anatomie et de la Physiologie, 1895.

between which at one pole of the egg there is a mass of scattered cells destined to become the permanent ectoderm. The outer layer of cells has a tendency to grow into the form of villi over the embryonic disc, while on the opposite side of the



FIGS. 1-3. — Diagrams of the Development of *Pteropus Edulis*, after Selenka. Fig. 1 is Selenka's Fig. 2; Fig. 2, Selenka's Fig. 5; Fig. 3, Selenka's Fig. 9. *R*, Rauber's layer; *P*, placenta; *ec*, ectoderm; *em*, entoderm; *cd*, chorion; *am*, amnion; *uv*, umbilical vesicle; *mes*, mesoderm; *co*, coelom; *all*, allantois, with the arrow indicating the direction of its future development.

egg it is composed of but a single layer of cells. Since this outer layer remains well separated from the body of the embryo throughout its development, and since it holds the same position to the egg that Rauber's layer does in the rodents, I

believe it to be identical with Rauber's layer, and shall speak of it as such. According to Duval this Rauber's layer disappears over the embryonic disc in the Chiroptera much as in the development of the rabbit and the field mouse. This does not necessarily contradict Selenka's observations on *Pteropus*, for the house mouse begins to develop like the field mouse, but continues during the early stages in the same manner as *Pteropus* does.

In the next stage the ectoderm has been converted into a hollow mass of cells, Fig. 2, rather by a process of absorption than by an invagination, as I have expressed it in the diagram. The entoderm lines the whole interior of the egg, and surrounds the ectoderm of the amniotic cavity. The ectoderm of the exterior of the egg, Rauber's layer, is again thickened over the embryonic mass to form the placenta, as Selenka calls it, or the *Träger*, if we were discussing rodent embryology.

In the next stage, as expressed in Fig. 3, the mesoderm is beginning to form, and has extended completely over the amnion and partly over the umbilical vesicle. The entoderm has retracted itself and touches the ectoderm; only the chorda dorsalis is yet to form. Between the amnion and the placenta, or the *Träger* portion of Rauber's layer, there is a marked space, and the mesoderm does not come in contact with it. The allantois grows as a bag into this space and attaches itself to the thickened part of the ectoderm, as shown by Göhre¹ in his figures. In the figure 3 accompanying Göhre's paper he shows the vesicular allantois attached to the support of the chorion (black portion of my Fig. 3) leaving on either side of the embryo a coelom. The allantois carries the mesoderm and vessels to the villi of the chorion, and these in turn are imbedded in the decidua of the uterus. In so doing the ectoderm of the chorion receives a second layer of epithelium, and I believe that this must account for the two layers of epithelium we have on the chorionic villi of the human ovum. There has been much written on the subject of the double layer of epithelial cells of the human chorion, and I think that a glance at Göhre's figures 3 and 4, on *Pteropus*, as well as at Selenka's figures 11

¹ Göhre: *Selenka's Studien*, etc., 1892, p. 218.

and 12 (Plate XXXV) and figure 6 (Plate XXXVII) on monkeys, will decide this question more definitely than all the many discussions on the human chorion put together have done.

Having now selected from Selenka diagrams and descriptions of the development of the germ layers of *Pteropus*, it is easier for me to give a plausible explanation of the beginning of the coelom in the human embryo. If the diagram I have given in Fig. 3 is compared with Selenka's figures 5 and 11 (Plate XXXV) and figure 5 (Plate XXXVII) of the monkey, as well as with the sections of young human ova published by Graf Spee¹ and by myself,² one is struck with the great similarity of the two groups of figures.

Fig. 14, given further on, is a diagrammatic outline of a longitudinal section of a young human embryo published recently by Graf Spee. It is the one marked v. H. in the table of young human ova given in the beginning of this paper. When, now, this section is compared with the transverse section of *Pteropus*, in Fig. 3, the only marked difference is that the umbilical vesicle in *Pteropus* has retracted, in order to make the arrangement of the membranes as given for the human embryo in Fig. 14.

In order to make the connection complete, I give hypothetical stages in Figs. 4, 5, and 6. Fig. 4 represents the human ovum in the two-layer stage. The outer layer, or Rauber's layer, is complete as in the rodents and in *Pteropus*. The inner layer, or entoderm, is also complete. Between the two is the embryonic shield, or ectoderm of the future embryo. The next figure, 5, shows the beginning of the mesoderm developing towards the tail end of the embryo, as this is the position of the primitive streak, and as the head fold of the amnion in many embryos is often only invested with ectoderm and entoderm. A stage later, Fig. 6, finds the mesoderm enveloping the umbilical vesicle completely, and also partly lining the outer layer, R, of the ovum. The cavity between the two is the coelom. At the tail end of the embryonic disc the mesoderm

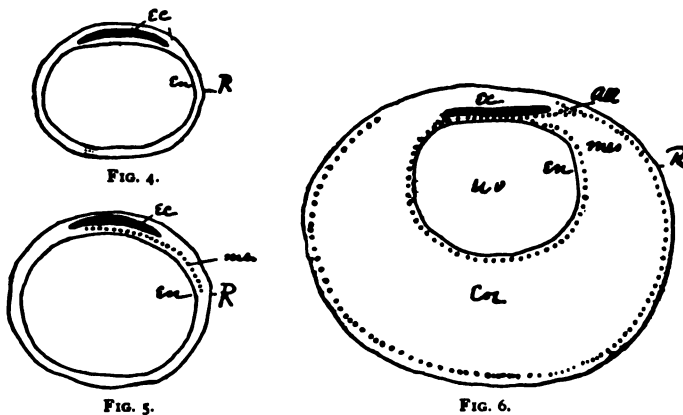
¹ Graf Spee: *His's Archiv*, 1889 and 1896.

² Mall: *A Human Embryo of the Second Week*, *Anatom. Anz.*, Bd. 8, and *Early Human Embryos and the Mode of their Preservation*, *Johns Hopkins Hospital Bulletin*, 1893.

of the somatopleure and splanchnopleure are still united, and mark the place of the formation of the rudimentary allantois.

Having carried the development of the human ovum to this stage by means of hypothetical stages, based upon the development of *Pteropus*, I can now continue the description of the development based upon observation.

Abnormal Ova. — Teratologists are accustomed to view a group of abnormal states as arrested development, and in recent years a number of abnormal human ova have been studied by His,¹ by Giacomini,² and others. Frequently in the



FIGS. 4-6. — Hypothetical Stages of the Early Development of the Human Ovum. *R*, Ranber's layer; *ec*, ectoderm; *en*, entoderm; *mes*, mesoderm; *uv*, umbilical vesicle; *coe*, coelom; *all*, position of allantois.

development of an ovum the embryo is destroyed completely, or, according to Giacomini, may wander out of the ovum. In these cases the ova are aborted. Frequently, however, a portion of the embryo is not developed, or it dies and the remaining portion develops for a time, and then the ovum is aborted. I have now in my collection a beautiful example of an ovum of apparently normal structure, the interior of which is lined completely with an amnion, and in place of an embryo

¹ His: Anatomie mensch. Embryonen, Heft 2, 1882, and Internationale Beiträge zur wissenschaftlichen Medecin, Bd. 1, 1891.

² Giacomini: Ergebnisse der Anatomie und Entwicklungsgeschichte, Bd. 4, 1895. The original papers of Giacomini are in the Archives Italiennes de Biologie, vols. XVIII-XXII.

there is only an umbilical cord. The ovum was aborted fifty-four days after the first lapsed period, and was 30 mm. in diameter. The cord was 2 mm. in diameter and 9 mm. long. Its embryonic end seemed to be cut off abruptly, and was covered with a small mass of round cells. I give this example only to show that the embryo may be entirely wanting with a perfect cord and membranes.

A large per cent of young ova which come into the embryologist's hands are abnormal. According to Professor His's

TABLE OF ABNORMAL OVA.

No.	DIAMETER OF OVUM.	DIAMETER OF EMBRYONIC MASS.	FROM WHOM OBTAINED.
XIII	8 mm.	1.4 mm.	Prof. His's No. XLIV, Leipzig.
XIV	30 "	1.5 "	Dr. Friedenwald, Baltimore, Md.
XX	15 "	2. "	Dr. Williams, Baltimore, Md.
XXI	12 "	5. "	Dr. Cullen, London, Canada.
XXXVII	25 "	2. "	Dr. Gould, Philadelphia, Pa.
LVIII	20 "	6. "	Dr. Howard, Cleveland, Ohio.
XXXII	30 "	2x9 "	Dr. Booker, Baltimore, Md.
XXIV	20 "	—	Dr. Miller, Baltimore, Md.
XXIX	30 "	—	Dr. Booker, Baltimore, Md.
LV	30 "	—	Dr. Watson, Baltimore, Md.

It does not necessarily follow that these embryos are all less than six weeks old, for the menstrual history of the mother indicates that some of them must be considerably older. This is one source of error in obtaining the high per cent of abnormal ova among young embryos. The statistics will not be accurate until the menstrual history accompanies the measurements.

experience over half of the ova less than three weeks old are abnormal, while of those of four and five weeks one quarter are abnormal. In my collection there are ten abnormal ova among twenty-six ova which are less than six weeks old. Of these ten specimens three contained no embryos at all, one (No. XXXVII) contained the cord only, and six were of the nodular form. Of this group of six, three contained a double vesicle with a kind of fibrous capsule, to a great extent similar to the mesoderm of the chorion. One of these is His's Embryo XLIV, which is frequently described in the books as a normal

specimen, but which unfortunately is an abnormal one. My interpretation of these three specimens (Nos. XIII, XIV, and XX) is that the fibrous degeneration overtook the embryonic vesicle after it had reached the stage of Graf Spee's embryo v. H., my Fig. 14. The remaining three embryos (Nos. XXI, XXXVII, and LVIII) are of the vesicular form, and I believe them to be especially valuable for the proper interpretation of the early stages of development of the human coelom.

Nos. XXI and LVIII came to me as perfect specimens, both having been hardened unopened, the first in strong formalin and the second in strong alcohol. No. XXI was still enclosed in its decidua, and appeared to be a normal specimen until it had been cut into serial sections. The embryonic vesicle proved to be very large, and was composed throughout of two layers, an inner one giving all the appearance of the entoderm and an outer giving all the appearance of the mesoderm of the umbilical vesicle of young embryos. The mesodermal layer contained within it islands of blood cells, as are also present in normal specimens. The whole vesicle was connected to the chorion with a mass of mesodermal cells somewhat as shown in the diagrammatic Fig. 7. The chorion and decidua appeared to be normal.

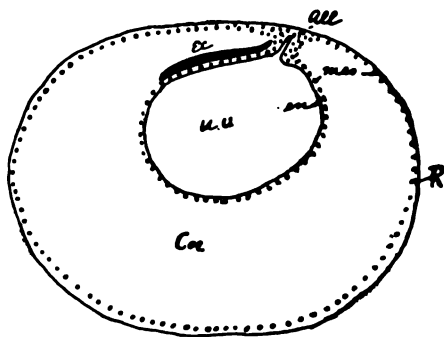


FIG. 7. — Diagram of a Pathological Ovum which represents an Early Hypothetical Stage.

No. LVIII showed considerable change in the mesoderm of the vesicle and chorion, giving somewhat the appearance of fibroid degeneration rich in cells. The chorion was attached to the vesicle by a strong pedicle, as shown in Fig. 7. The vesicle itself was composed of two layers, an inner and continuous one composed of one layer of cells, and an outer and thickened layer appearing like the mesoderm of the chorion. There were no indications of blood islands. In addition to these two layers there was a third layer fairly well marked near

the pedicle and between the vesicle and the chorion. With the exception of the allantois canal, Fig. 7 is a diagram of this specimen. No. XXXVII is much like No. XXI, but it did not stain well as the specimen was a number of years old when cut.

Giacomini¹ has described a number of similar vesicles, and he expressly states that the vesicles had the structure of the

umbilical vesicle, but that there was no trace of the amnion present in any of them. A number of other vesicular forms have been described, and in general they all appear much like the two specimens I have given.

I do not think that it is rash to assert that these vesicles represent an arrested development of an earlier stage, which, due to impaired nutrition, or whatever it might have been, simply allowed the embryonic vesicle to keep on expanding. That this expansion can keep on is already shown in the sim-



FIG. 8.—Diagrammatic Section of Half of the Human Ovum No. XI. Enlarged 10 times. The villi are drawn only on the upper side. *ex*, ectoderm; *en*, entoderm; *mes*, mesoderm; *u*, *v*, umbilical vesicle; *coe*, coelom; *all*, allantois; *a*, amnion.

ple enlargement of the chorion after the embryo is distorted or wanting altogether. We have in these specimens a thin chorion with atrophic villi, and why can we not have an expanded and atrophic embryonic vesicle if its development is impaired? In this way I view specimen No. LVIII. It represents a much earlier stage, which has simply expanded and was ultimately aborted. In No. LVIII the embryonic vesicle must have ceased its further development a week or so before the abortion, about the time the coelom was beginning to

¹ Giacomini: *Ergebnisse d. Anat. u. Entwicklungsgesch.*, Bd. 4, S. 636.

develop. At that time the fibrous degeneration enclosed the embryonic vesicle as well as extended around the whole chorion into all of its villi. This, then, arrested the further development of the embryo, and the embryonic vesicle simply continued to expand.

This idea is further strengthened by another ovum whose history I published on several occasions three years ago.¹ The specimen is a good one, having been preserved fairly well, and it has every indication of being normal. Since the specimen has been in my hands I have studied it over and over again, have photographed many of the sections, and have reconstructed it. At first it was very difficult for me to interpret it, but finally it appears to me that something definite can be said regarding the arrangement of the membranes and their relation to older as well as to the pathological and presumably younger specimens.

Embryo No. XI.—"The woman, from whom the specimen was obtained, is twenty-five years old, menstruates regularly every four weeks, the periods lasting from four to five days. She gave birth to a child Sept. 19, 1892, and had the first recurrence of menstruation December 19. The second period followed on January 25, and was very profuse; it lasted until February 1. The next period should have begun about February 22, but on account of its lapsing the patient concluded that she was pregnant, and called at my office a few days later. I did not examine her, but asked her to remain quiet and await developments, as I thought possible that she might be pregnant. On the evening of March 1 she fell and sprained herself, and during the same night had a scanty flow. The flow recurred each day, and on the 7th of March she passed the ovum. It was kept in a cool, moist cloth for twenty hours, and when it came into my hands was at once placed in a large quantity of 60% alcohol."²

The ovum is very large for its age, having a long diameter of 10 mm. and a short diameter of 7 mm. It is covered with villi only around its greatest circumference, having two spots with-

¹ Mall: *Anatom. Anz.*, 1893, and *Johns Hopkins Hospital Bulletin*, 1893.

² Letter from Dr. Kittredge, April 27, 1893.

out villi, as was the case with Reichert's ovum. The villi of the chorion are from 0.5 to 0.7 mm. long and are branched.

Upon opening the chorion it was found that the germinal vesicle was situated just opposite the edge of the zone of villi. About it was much coagulated albumen, which I did not remove, and therefore could not obtain good camera drawings. The portion of the chorion to which the vesicle was attached was cut out and stained with alum cochineal and cleared in oil, but even after this treatment it was impossible to obtain any clear picture. The specimen was next imbedded in paraffin and cut into sections 10 μ thick. The series proved to be perfect. From the sections a reconstruction was made in wax, and the accompanying Fig. 8 is a sagittal section of it.

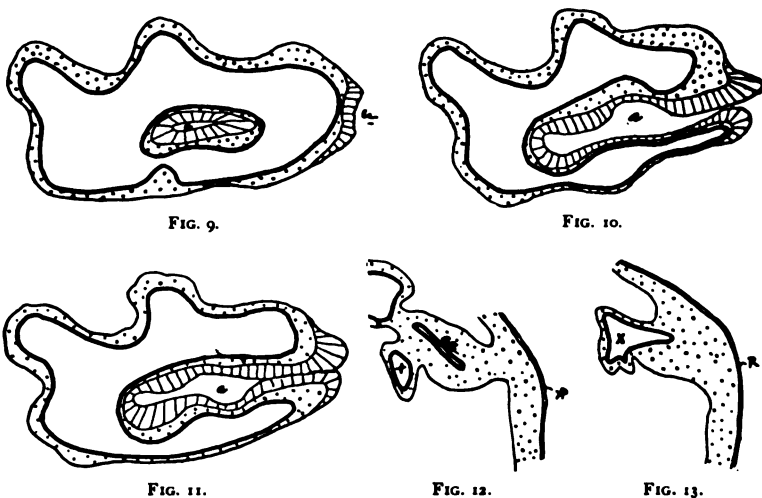
The dimensions of the different portions of the vesicle are as follows:

Diameter of stem	0.4 mm.
Length of stem	0.4 "
Length of vesicle	1.5 "
Width of vesicle	1.0 "
Length of invagination	0.8 "
Width of invagination	0.5 "
Diameter of opening of invagination	0.03 "

The sections and reconstruction show that the embryonic vesicle is attached to the chorion by means of a stem (*Bauchstiel*). The vesicle itself is composed of two layers, between which, at a distance from the stem, there are indications of blood-vessels in the middle embryonic layer. Just beside the attachment of the vesicle to its stem there is a deep, narrow invagination of all layers of the vesicle. The walls of the invagination are somewhat thicker than those of the surrounding vesicle. The accompanying figures give the arrangement of the embryonic layers in different portions of the vesicle. The invagination is in no respect artificial, as suggested by Graf Spee, as the curves are all sharp, and the layers of mesoderm and ectoderm are very definitely outlined. The ectoderm has the sharp contour of the ectoderm of other young embryos published, and gives the pictures which are familiar to all embryologists. The entoderm does not extend all around the sections as I have pictured it, but has fallen off at some points, and

this explains why the figures here given do not correspond exactly with those in previous publications. There cannot be any doubt about my interpretation of the arrangement of the embryonic layers in this specimen, nor do I think that it is abnormal. Yet this last point will be decided in the near future, I believe, and therefore it may be dropped until other young embryos are described.

Within the stem of the vesicle there is a sharply defined allantois, which communicates with the cavity of the vesicle



Figs. 9-13. — Sections No. 43, 53, 68, 80, and 89 through the Embryonic Vesicle of Embryo No. XI. Enlarged 33 times. The entoderm is a heavy line, the ectoderm is striated, and the mesoderm dotted. *a*, amnion; *X*, cavity of the umbilical vesicle extending into the stem of the vesicle; *R*, Rauber's layer as the ectoderm of the chorion.

just below the invagination of the ectoderm. The cavity of the vesicle extends into the stem at a lower point, and it is this invagination which Graf Spee believes to be the amnion. Gladly would I agree with him were it not that there is no evidence whatever of the presence of ectoderm at this point. Throughout this invagination into the stem there are only two thin layers of cells, one of which runs over into the mesoderm of the chorion and the other into the entoderm of the vesicle. Yet in this invagination the entoderm is not detected at any point.

The ectodermic plate in the large invagination of the amnion is very broad, but not of equal thickness throughout its extent, and it ends very abruptly beyond the opening upon the surface. As the opening approaches the stem, the cells of the ectoderm are continued somewhat along its surface, as indicated by the black line in Fig. 8.

All of the space between the embryonic vesicle and the chorion is the coelom, and in this specimen it communicates with the amnion. Whether this is transient or unusual cannot, of course, be stated. Should further experience show that the amnion is closed at an earlier stage than indicated in this specimen, it would not materially affect my diagrams or observations. Graf Spee's recent observation, Fig. 14, makes him think that this is the case, but it is just as easy to interpret the formation of the amnion in Fig. 14 from that in Fig. 8 as by his theory.

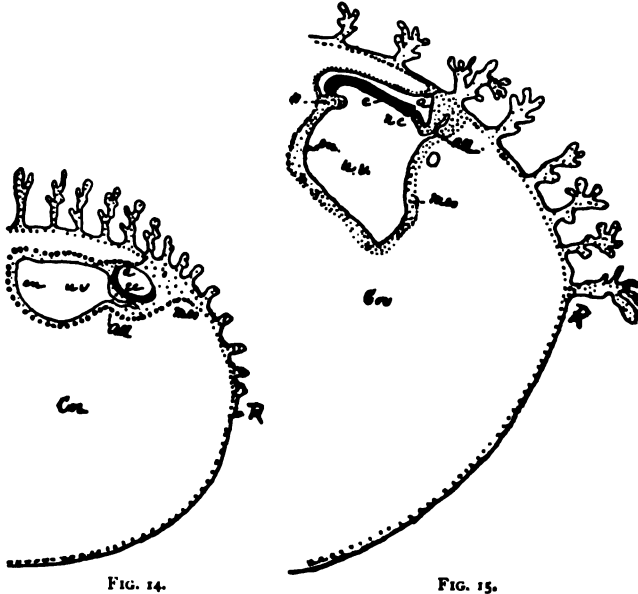
The next stages in the development of the embryonic vesicle are taken from Graf Spee, and they are of importance to elucidate the changes which take place preparatory to the formation of the body cavity. In Fig. 14, which represents the younger embryo, the amnion is still surrounded completely with mesoderm, as in embryo No. XI, represented in Fig. 8. The mesoderm crosses the median line, as the sections given by Graf Spee¹ show. The dorsal side of the amnion is covered with a very thick layer of mesoderm, as the closure of the amnion in embryo No. XI would suggest.

From the stage represented in Fig. 14 it is easy to pass to the older embryo represented in Fig. 15. Now the body of the embryo is well marked, the neural folds are just beginning, and the neurenteric canal has just been formed. The chorda dorsalis is not yet separated from the entoderm, and the blood islands encircle completely the umbilical vesicle and have nearly reached the head end of the body of the embryo preparatory to the formation of the heart.

It is not very difficult to imagine the embryonic vesicle of Fig. 8 to be converted into the vesicle of Fig. 14. To be sure, the invagination in Fig. 8 seems to be much larger than neces-

¹ Von Spee: *His's Archiv*, 1896, Plate I, Figs. 4, 5, 9, and 10.

sary, but variations of this kind are frequently encountered in the study of embryology. In the diagrammatic outline of von Spee's embryo v. H. (Fig. 14), I have emphasized the variation of the thickness of the ectoderm lining the amnion to correspond with von Spee's Figs. 7, 8, 9 in his recent publication.



FIGS. 14 and 15. — Longitudinal Sections of Two Young Human Ova, after Graf Spee. Enlarged 10 times. Fig. 14, Embryo v. H.; Fig. 15, Embryo Gle. Just half of the chorion is drawn, and the villi are outlined only over a portion of the ovum. *R*, Rauber's layer; *a*, amniotic cavity; *uv*, umbilical vesicle; *en*, entoderm; *mes*, mesoderm; *all*, allantois; *c*, chords; *nc*, neurentic canal; *H*, position of heart.

His longitudinal section, from which my figure is taken, does not emphasize this point, which I consider of importance in this discussion.

In these two ova described by von Spee, the coelom is much of the same form it was in embryo No. XI, Fig. 8, and therefore needs no special comment. Yet around the head end of embryo Gle. there is a marked accumulation of mesoderm into which the heart is to grow. In the illustrations of the section of this embryo Graf Spee¹ pictures spaces in the mesoderm which he believes to be portions of the body cavity of the

¹ Graf Spee : *Hls's Archiv*, 1889.

embryo, that is, the cavity of the muscle plates, pericardial cavity or peritoneal cavity. It is impossible to determine definitely which portion of the body cavity these spaces represent, but I do not feel inclined to believe that what he marks pericardial cavity in Fig. 23 can possibly represent it, for we are to look for the pericardial cavity between the junction of the pharynx and umbilical vesicle and the head end of the embryo. This portion of the embryo is marked H in my Fig. 15, and falls anterior to von Spee's Fig. 16. Von Spee's Fig. 16 is the 24th section of the embryo, beginning at the head, while his Fig. 23 is the 81st section.

The various small spaces in different portions of the mesoderm cannot be viewed as the real origin of the body cavities without further discussion. In the von Spee embryo v. H. there are indications already of small spaces in the mesoderm at the border of the ectoderm of the embryo. Similar spaces are described by Bonnet¹ for the sheep and by Selenka² for the monkey. While von Spee and Bonnet believe that these spaces belong to the coelom, Selenka simply designates them heart, or vascular.

The blood-vessels are intimately associated with the coelom in their early development, and it is easy to be led into error without an abundance of material. Drasch³ recently has again emphasized this relation. He has shown in the chick that the blood islands are separated from one another by a number of closed spaces filled only with a fluid. These spaces soon flow together to form the large slit-like coelom of birds. The same condition of things has been shown to be true, but from a very different method, by Budge.⁴ He injected the blastoderm of the chick, and showed that the coelom was composed of a network of spaces, which gradually flowed together into the large coelom surrounding the embryo.

Of course in the young human embryos we have at our disposal this stage of the process has long passed, but there is no reason why a remnant of it should not exist at the point of

¹ Bonnet: *His's Archiv*, 1889.

² Selenka: *Studien*, etc., *Taf. XXXVIII*, Fig. 35.

³ Drasch: *Anatom. Anz.*, Bd. 9.

⁴ Budge: *His's Archiv*, 1887.

union of the umbilical vesicle with the body. The reason I question von Spee's interpretation of these small spaces in the mesoderm in embryo Gle. is that I believe that all, or certainly nearly all, of the body cavity is formed by an incorporation of the extra-embryonic coelom within the embryo. What I have observed in human embryos as well as in the injected specimens of Budge shows that this must be true. These small spaces in the mesoderm of the body may belong to the muscle plates and the early blood-vessels, and certainly cannot play any great part in the development of the body cavity. There is no doubt whatever that the whole peritoneal cavity is simply pinched off from the coelom of the outside of the body and it is highly probable that the pericardial cavity and pleural cavities are formed in the same way. The anterior mesentery of the intestine has never existed in the human embryo, and it is therefore needless to explain its mode of disappearance.

My statements are based in great part on embryos Nos. III and XII, and since No. XII is such a perfect specimen it is well for me to describe it in greater detail. The embryo is about the same age as Kollmann's¹ embryo Bulle, which unfortunately was never fully published. No. III is an embryo given me by Professor His. This embryo had been torn from the umbilical vesicle, and was injured in different portions of the body. Yet the head end of it is fairly well preserved, and it is of value in determining the growth of the body walls covering the heart.

Embryo 2.1 mm. long. — The history of embryo No. XII is as follows. "The woman from whom the ovum was obtained is twenty-three years of age and has been married for three years. She is a very intelligent woman, and her statements are reliable. Her menstrual periods recur every thirty days. She had been married some time before she became pregnant, and after passing two periods aborted July 6, 1893. She was unwell the 5th of October and again on the 7th of November, this last period

¹ Kollman: His's Archiv, Supplement Bd., 1889, Plate V, Figs. 1 and 2; von Lenhossék: His's Archiv, 1891, Plate I; Kollman: His's Archiv, 1891, Plate III, Fig. 3.

lasting five days. She passed her next period and on December 18th aborted the ovum."¹

The ovum was hardened in strong alcohol without opening it first, and when it came into my hands its dimensions were 18

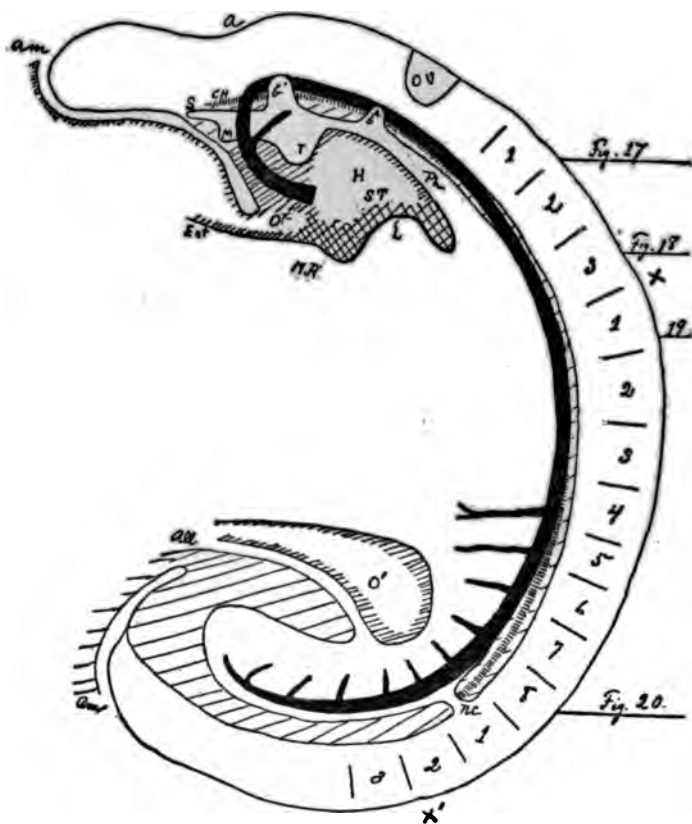


FIG. 16. — Outline Drawing of a Sagittal Section of the Model of Embryo No. XII. Enlarged 50 times. The heavy line is the aorta. The muscle plates are numbered for occipital, cervical, and dorsal regions, respectively. The mesoderm is striated. *am*, amnion; *a*, border between fore-brain and mid-brain; *x* and *x'*, extent of closure of spinal canal; *S*, Seessel's pocket; *ch*, chorda; *b'* and *b''*, first and second branchial pockets; *o v*, otic vesicle; *m*, mouth; *T*, thyroid; *H*, pericardial space; *ph*, pharynx; *ent*, entoderm; *S T*, septum transversum; *l*, liver; *n c*, neurenteric canal; *all*, allantois.

× 18 × 8 mm., that is, it was slightly flattened. It was completely covered with long villi. It was carefully opened, care having been taken not to injure the embryo in any way. The

¹ Letter from Dr. Ellis, Jan. 7, 1894.

coelom was filled with a clear fluid, and many firm shreds of a fibrine-like body which obscured the embryonic vesicle greatly. With much difficulty the embryo could be outlined, and these drawings proved to be of great service in making the reconstruction. The portion of the chorion to which the embryo was attached and the embryo were stained in carmine and imbedded in paraffin. The whole was cut into sections, at right angles to the body, 10 μ thick.

Every other section was enlarged 100 times and drawn on wax plates 2 mm. thick, and from them the model of the embryo was made. The model gives the whole central nervous system, the entoderm throughout its extent, the blood-vessels, and the muscle plates.

The shape of the neural tube is given in the diagrammatic outline. It was closed only along the middle of the body, being open in front down to the beginning of the fourth muscle plate. From the beginning of the fourth plate to the beginning of the fourteenth it was closed, and from there on again it was open. In the figure the portions between x and x' indicate to what extent the tube is closed. In Figs. 17 and 18 the tube is nearly closed, while in Fig. 20 the tail end of the tube is just beginning to separate from the ectoderm. The cephalic end of the tube already clearly outlines the fore-brain, the mid-brain, and the hind-brain; the constriction, Fig. 16, a , indicates the junction between the first two. On the ventral side of the fore-brain there are two marked pockets, one on either side, just behind the neuropore, which are no doubt the primary optic vesicles. It shows that in the human embryo these are fully outlined before the brain has separated itself from the ectoderm. Farther behind, very near the dorsal median line and about in the middle of the head, there is a short pocket of thickened ectoderm, the otic vesicle. Towards the hinder end of the embryo the spinal cord communicates by means of a solid band of cells with the entoderm, Fig. 20. At no point in this communication is there a canal, so it must be viewed as the last remnant of the neurenteric canal. The location is opposite the twelfth muscle plate, or in the neighborhood of what will later on be the position of the first rib. The chorda

dorsalis extends to the neurenteric canal, but not beyond it. There is no chorda in the tail end of the embryo.



FIG. 17.



FIG. 18.



FIG. 19.



FIG. 20.

FIGS. 17-20. — Sections through Embryo No. XII, as indicated by the Lines in Fig. 16. Enlarged 50 times. The black is the coelom within the body. O^1 and O^3 , first and third occipital muscle plates; C^1 and C^8 , first and eighth cervical muscle plates; D^1 , first dorsal muscle plate; a , aorta; v , omphalomesenteric vein; t , thyroid; l , liver; ph , pharynx; i , intestine; nc , neurenteric canal; mr , membrana reuniens.

Throughout the central nervous system, immediately about the central canal, there are many karyokinetic figures, showing

that the specimen was excellently preserved. In the greater portion of the neural tube the tissue is already marked by two zones, a central one rich in nuclei, and a peripheral containing none. This corresponds with the description already made familiar to us by His.

The general shape of the whole central nervous system is very unlike that of any other young human embryo ever published. It circumscribes the greater portion of a circle, while in the other human embryos of this size it makes more of a straight line. I think that it is probable that this specimen represents the normal, as it was not injured nor handled in any way before it was cut into sections.

The entoderm, as the figures show, is already divided into fore-gut, mid-gut, and hind-gut. The fore-gut makes the pharynx, from which there are four diverticula on the dorsal side, one on the ventral side, and two near the mouth. The four on the dorsal side mark the first two branchial pockets on either side of the embryo ; the two in front are Seessel's pocket and the entodermal portion of the mouth ; while the one on the ventral side of the pharynx is the beginning of the median portion of the thyroid gland (Fig. 17, t.).

At the junction of the pharynx with the umbilical vesicle there is a large diverticulum into the septum transversum, Fig. 18 l, the beginning of the liver.

Within the tail end of the embryo, behind the neurenteric canal, the hind-gut is enlarged considerably, and from it the entodermal canal of the allantois arises.

The whole umbilical vesicle is covered with blood-vessels which communicate with the veins and arteries of the embryo. Near the origin of the liver there are two veins which collect the blood from the umbilical vesicle and then enter the heart. These are the omphalomesenteric veins. They with a number of their branches are shown in sections in Fig. 18, v. The heart itself is broken, but there is enough of it left to show that it is bent upon itself and contains a large cavity at the point where the veins entered it. From the heart two arteries arise and pass in front of the first branchial pocket, and each follows the course as shown in black in the reconstruction.

The aortae do not unite, but each sends a number of segmental branches to the umbilical vesicle along the tail end of the embryo. These are, of course, temporary; they may be called collectively the omphalomesenteric arteries. As the permanent omphalomesenteric artery arises more aboral than any of these, it is easy to understand that most of them must degenerate.

The sections show that there are fourteen muscle plates, all of which are hollow and do not in any way communicate with the body cavity in general. Kollman, who described an embryo of this same age, numbers them from before backward, but I think that they can be designated more definitely. Froriep¹ showed that in all amniotic vertebrates there were a number of muscle plates and dorsal ganglia formed in the occipital region, and studied their fate in the chick and in the cow's embryo. Platt² has also followed the order of the origin of the muscle plate in the chick, and found that the first division of the mesoderm was between the third and fourth occipital plates. The first three or four of these segments communicate in the chick, according to Dexter,³ with the coelom, and Bonnet⁴ has found also that the same is true in the sheep. Bonnet's figures (compare his Plate IV) show that a sheep's embryo of the same stage as embryo XII has muscle plates much more sharply outlined than the human. In order to locate the muscle plates more definitely I have made every effort to count the spinal ganglia in embryo XII, but with no definite result. It is impossible for me to define the spinal ganglia, as often they are represented by a few cells only, then again as a band of cells they extend over several segments. The same is true in the occipital region. Had I been able to number them definitely it would still have been impossible to number the muscle plates from them, for His⁵ has shown that there is an occipital ganglion in the human embryo as well as in the lower animals.

The fact that the muscle plates reach to the otic vesicle in

¹ Froriep: *His's Archiv*, 1883 and 1886.

² Platt: *Bulletin of the Museum of Comparative Zoölogy*, vol. XVII.

³ Dexter: *Anatom. Anz.*, 1890.

⁴ Bonnet: *His's Archiv*, 1889.

⁵ His: *Abhandl. d. säch. Gesellsch. d. Wiss.*, Bd. XXIV.

embryo XII, as well as in Kollman's embryo Bulle, indicate that the first plates must belong to the occipital region, and I have found that there are three occipital muscle plates in embryo No. II.¹ Moreover, there is every indication of a degeneration of the first two plates in XII, so on this account I am inclined to number them as they are numbered in Fig. 16. I do not think that any of them ever communicate with the pericardial cavity as Bonnet found them in the sheep. The cavities in all of the other plates are small, and they are separated by a large mass of mesoderm from the coelom. This all confirms my view.

The chorda extends from Seessel's pocket to the neurenteric canal.

There are also a few segmental ducts, some completely and some partly separated from the ectoderm, as was the case in Kollman's embryo. The ducts are small, and extend over one or two sections only, and occasionally one of them is arising at several different points between a given two segments. They are present on both sides between the first and second cervical segments, second and third segments, third and fourth segments, fourth and fifth segments, and only on the left side in the region of the fifth and sixth cervical segments.

The coelom of this embryo is especially instructive. A sagittal section of the embryo and ovum is given in Fig. 21. This embryo, when drawn connected with the ovum, is very similar to Graf Spee's embryo Gle. as shown in Fig. 15. It is very easy for us to conceive the von Spee embryo converted into this embryo, for about all the change that is necessary is that the embryo grow somewhat and bend upon itself. In so doing the attachment of the umbilical vesicle becomes smaller as the amnion encircles the body of the embryo more. The position of the neurenteric canal, the shape of the allantois, and the formation of the pericardial cavity, all show that the curving must be a normal one.

Nearly all other young embryos of this stage, or a little older, which have been published show a straighter body or even a curve in the opposite direction. I have also in my collection

¹ See also Mall: *Journ. of Morph.*, vol. V.

two embryos of this stage, Nos. I and XV, which had been taken out of the chorion and torn from the umbilical vesicle, and both of them are straight like Kollman's embryo Bulle and His's¹ embryo L. It is difficult to conceive how my embryo XII could possibly be torn out of its membranes without straight-

ening it. We need only recall our experience in hardening embryos of lower animals to be reminded how easily a curved embryo is straightened when it is handled the least bit roughly before it is hardened.

His, in his great monograph on human embryos, emphasizes a curve in the back of the embryo just the reverse of the one given in Fig. 21. I refer to embryos Sch., BB., and Lg., as well as to Minot's embryo 195.² The fact that this inverted bend in the back is not constant (His's Rf., for instance), and that it

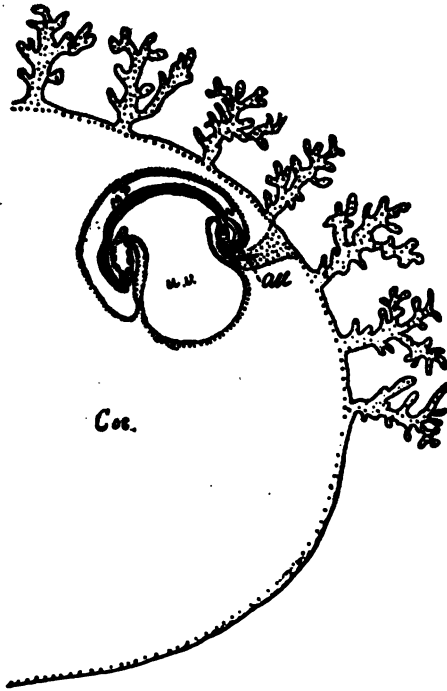


FIG. 21. — Sagittal Section of the Ovum with Embryo No. XII Attached. Enlarged 10 times. *Co.*, coelom; *uv*, umbilical vesicle; *all*, allantois; *mp*, medullary plate; *nc*, neurenteric canal.

occurs at the time when any tension upon the umbilical vesicle could produce it, makes me believe that it is an artifact. This view was suggested to me a number of years ago, when I was removing young dogs' embryos from the uterus, and unwittingly distorted a number of them in this very way before they were hardened. The middle of the back is the weakest part of the embryo's body, and the umbilical vesicle is attached to

¹ His: *Anat. mensch. Embryonen*, Plate VI.

² Minot: *Human Embryology*, New York, Fig. 169.

it. Under these conditions the simple weight of the vesicle is sufficient to bend the back of the embryo as pictured by His.

To return to the coelom. At the hinder end of the embryo the coelom dips into the body overlapping the hind-gut in the neighborhood of the neurenteric canal, as shown in Fig. 20. This cavity communicates with its fellow on the opposite side through an opening between the umbilical vesicle and the allantois, marked O in Fig. 16. This communication has already been described by His¹ for an embryo somewhat older. If, now, the point O in Fig. 16 is approximated towards NC, with a flexion of the embryo at the same time, this communication is easily explained. In other words, as the hind-gut is being separated from the umbilical vesicle, a groove-like portion of the coelom is also included in the body of the embryo. At the hinder portion of the embryo, on either side, the coelomic grooves extend deeper into the body of the embryo, and communicate with each other around the aboral side of the stem of the umbilical vesicle. This communication is shown well by His in Fig. I, B, Plate VI of his *Atlas*, as well as in the same figure, page 299 of Minot's *Embryology*. Excellent profile views showing this point are given in all the embryos figured on Plate IX of His's *Atlas*.

I emphasize this point in order to exclude the ventral mesentery for this portion of the embryo. The fact that this mesentery could never have existed in the human embryo is also proved by a careful examination of His's models of human embryos made by Ziegler.

As we pass towards the head in embryo XII the coelomic groove communicates freely with the extraembryonic coelom until the region of the membrana reuniens is reached. This is shown in Fig. 19, MR, with the membrana reuniens complete on one side, but not yet united on the other. The membrana reuniens extends up to the heart, and separates the pericardial cavity from the extraembryonic coelom, then crosses the ventral median line to return on the opposite side of the embryo. Throughout the extent of the membrana reuniens there is a great increase of mesodermal tissue, which encircles completely

¹ His: *Anat. mensch. Embryonen*, I, p. 126.

the beginning of the liver, as Fig. 18 shows. A portion of this mesodermal tissue has been described by His as the septum transversum.¹ According to His only that portion of the mesodermal tissue is septum transversum which lies between the posterior part of the pericardial cavity (*Parietelhöhle*), the wall of the intestine, and the point where the veins enter the heart. It extends across the body, and has within it the beginning of the liver. In transverse section this region is shown in Fig. 18. Now the pericardial cavity communicates by means of a long canal on either side, with the peritoneal cavity, and the omphalomesenteric vein hangs into this, attached to a kind of mesentery, as Fig. 18 shows. Lower down, near the communication (Fig. 19), there is an indication of the beginning of the umbilical vein, which unites with the omphalomesenteric vein through the membrana reuniens. The two canals which communicate with the extraembryonic coelom are the pleural cavities, and the membrana reuniens aids to separate them from the peritoneal.

All of the tissues from the diaphragm to the opening of the liver duct into the duodenum arise from the septum transversum and the membrana reuniens; the stomach from the fore-gut, the liver from the liver diverticulum, and the diaphragm from the septum transversum and the membrana reuniens. The Cuvierian duct must also have arisen in the membrana reuniens, in order to pass around the outside of the body cavity to reach the cardinal and jugular veins, as pictured by His² for the human embryo.

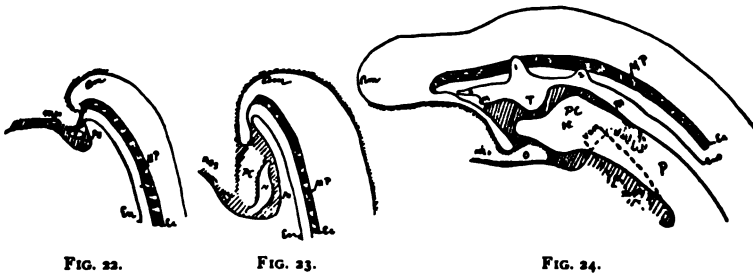
In the further development of the pleural and pericardial cavities the Cuvierian veins give us our best landmark, as they define the point where the pleural cavity is to be separated from the pericardial. And it really seems, as if the greater portion of the diaphragm is formed from the portion of the septum transversum on the ventral side of the vein and from the membrana reuniens, rather than from the portion immediately in front of the intestine. In other words, there is a

¹ His: *Anat. mensch. Embryonen*, I, p. 126.

² His: *His's Archiv*, 1881, Plate XII, Fig. 9. Also *Anat. mensch. Embryonen*, Plate IX, Figs. 10-12, 14.

horseshoe-shaped ridge of tissue around the neck of the embryo to the ventral side of the pericardial and pleural cavities and parallel to them. The median portion is composed of the septum transversum, and each wing of the *shoe* is the membrana reuniens, one on either side of the embryo. Its general direction in this stage is parallel with the long axis of the embryo, and within each wing there is an omphalomesenteric vein.

Origin of Pericardial Cavity.—With the pericardial cavity opening into the extraembryonic coelom on either side as a basis, it is possible to trace back the pericardial cavity to its



FIGS. 22-24. — Three Stages to show the Development of the Blastodermic Layers at the Head End of the Embryo. Fig. 22, Hypothetical Stage. Fig. 23, Embryo No. III. Fig. 24, Embryo No. XII. *V*, vein; *ph*, pharynx; *am*, amnion; *mp*, medullary plate; *pc*, pericardial cavity; *S*, Seessel's pocket; *m*, mouth; *t*, thyroid; *r* and *s*, first and second branchial pockets; *p*, pleural cavity; *mr*, membrana reuniens; *vom*, omphalomesenteric vein, which is expressed as a dotted line; *O*, communication between right and left body cavities on the ventral side of the umbilical vesicle.

origin. Figs. 16 and 24 show that the ventral wall of the pericardial cavity is composed mostly of mesoderm. This is the portion of the membrana reuniens which is composed of mesoderm, as the sections, Figs. 18 and 19, show. An earlier stage is shown in the diagrammatic Fig. 23. It is taken from embryo No. III. In this specimen, since the ectoderm of the amnion has not reached completely around the body, as both the sagittal and transverse sections show (Figs. 23 and 25), it is evident that the pericardial space is first covered on the ventral side with mesoderm and later the ectoderm is added when the amnion begins to close over the head. In embryo III the canals communicating between the pericardial space and the extraembryonic coelom are not as long as in embryo

XII, and the ventral walls of the pericardial space are composed wholly of mesoderm. This indicates that the growth of this wall was first by a union of the mesoderm, which was followed by the ectoderm of the amnion to complete the body wall. The process is shown in Figs. 22-24. Fig. 22 is a hypothetical stage between Graf Spee's embryo Gle. and my embryo No. III. As the process from Graf Spee's embryo continues, the blood-vessels reach the body to form the heart, as indicated by the outlines marked v. in Fig. 22. The mesoderm of the



FIG. 25. — Section through the Head of Embryo No. III. Enlarged 55 times. *Ph*, pharynx; *H*, heart. The arrow in the amniotic cavity indicates the direction of the future growth of the amnion to complete the ventral body wall.

amnion then unites with that of the umbilical vesicle, and the first pericardial space is formed. This is not wholly an imaginary stage, for it is based upon Bonnet's observations upon the sheep,¹ as well as Cadiat's upon the chick.² In a sagittal section of a sheep's embryo of about the same stage (Plate III, Figs. 16-20, c CB) Bonnet gives a similar fold, and after the pericardial walls are well formed he gives an illustration of a stage in which it still communicates with the extraembryonic coelom (Plate IV, Fig. 17, KC). With Graf Spee's embryo Gle. and with Bonnet's observations

upon the sheep as a starting-point, it is not difficult to interpret Figs. 22-24.

Extension of the Amnion. — After the stage of embryo XII is passed the amnion rapidly envelops the whole body and soon passes out over the cord. The next stage after No. XII which I have studied is No. XIX. I have very perfect photographs of this specimen, and the sections are all good, although the nervous system is macerated. The embryo has rotated in the amnion, throwing the cord to the right side with the left side towards the observer. It would have been impossible to obtain a view of the right side of the embryo without cutting the cord. The outlines of this embryo and ovum are given in

¹ Bonnet: *His's Archiv*, 1889.

² Cadiat: *Jour. de l'Anat. et de la Physiol.*, 1883, Plate V, Figs. 1, 2.

Fig. 26. Two sections through the body are given in Figs. 27 and 28.

The amnion has become separated from the body with the exception of the part about the cord and also that along the right side of the body, over the heart. The arrow in Fig. 25 shows how the amnion on that side is extended over the ventral body wall to make the condition shown in Fig. 28. No doubt the cause of this is the rotation of the body, throwing the cord to its right side and the amnion with it. In nearly all young embryos the cord is on the right side.¹ With the exception of the four instances mentioned below, the rotation has always been so as to throw the left side of the body away from the chorion, and in all of these specimens the amnion must have swept over the body from left to right, as shown in the figures. I find a similar illustration by His in his great monograph.²

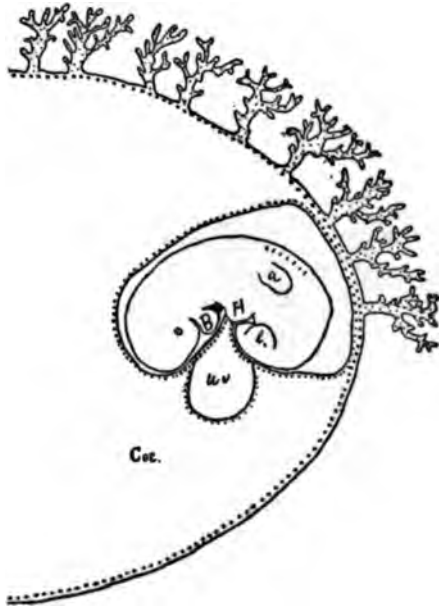


FIG. 26. — Ovum and Embryo No. XIX. Enlarged 5 times. Just half of the ovum is shown. *A*, arm; *L*, leg; *H*, heart; *u v*, umbilical vesicle; *B*, branchial arch.

Absence of a Ventral Mesentery. — After the septum transversum has been formed as it is in embryo XII, there is on its ventral side a pretty sharp groove, which indicates that the umbilical vesicle is being constricted at this point.

It is generally believed that the ventral mesentery of the intestine extends to the umbilicus, and that ultimately the round ligament of the liver represents its remnant after most of it has

¹ The exceptions have been published by Waldeyer: *Studien des physiol. Inst. zu Breslau*, 1865; Janošík: *Arch. f. mik. Anat.*, Bd. 30; His: *Anat. mensch. Embryonen*, Plate VIII, Figs. A 1-4; Mall: *Journ. of Morph.*, vol. V.

² His: *Anat. mensch. Embryonen*, Plate VI, Fig. 3, No. 10.

disappeared. This theory is expressed by two diagrams in Minot's *Embryology*, page 767. As the liver begins to grow, and while the heart is being pushed down in front of it, the ventral end of the septum transversum is turned down to the umbilicus. While this is taking place the stem of the umbilical vesicle becomes relatively smaller and smaller, but there is no union between the umbilical vesicle and the septum transversum as expressed in Minot's diagram. The first stage of this



FIG. 27.



FIG. 28.

FIGS. 27 and 28.—Section through Embryo XIX to show the Attachment of the Amnion to the Side of the Body. Enlarged 25 times. *Am*, amnion; *S*, stomach; *H*, heart; *c*, cardinal vein; *u*, umbilical vein; *a*, aorta.

process is shown in my Fig. 24, and its successive stages are shown in His's *Atlas*, Plate IX. In all six embryos pictured on that plate the successive stages are represented, and in none of them is the umbilical vesicle attached to the septum transversum to form a ventral mesentery. From these embryos of His we can pass to embryo XIX, in which the umbilical vesicle communicates by a round canal with the intestine, and the tube is completely encircled with a space which extends to the liver, thus cutting off any possible ventral mesentery at that point. The same thing is shown, but in a later stage, in

Fig. 30, *O*, but a new process has already taken place to complicate matters.

In embryo XII there is just a beginning of an umbilical vein in the membrana reuniens. In Kollman's embryo the vein is more marked.¹ The vein extends out into the somatopleure, far away from either the intestine or the median line. This same position is again shown in His's embryos BB. and Lr on Plate IX in his *Atlas*. The left umbilical vein becomes

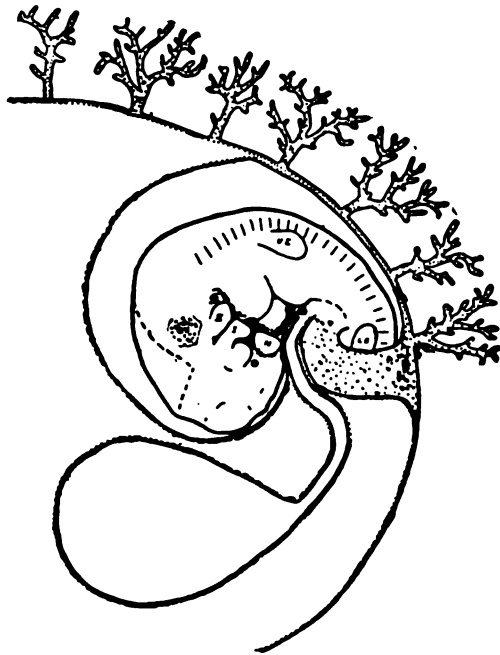


FIG. 29. — Embryo No. II Attached to the Chorion. Enlarged 5 times. Just half of the Ovum is shown. *O v*, otic vesicle; *U E*, upper extremity; *L E*, lower extremity; *N*, nose; *I, II, III*, branchial arches.

the more prominent, and as the body wall is developed more and more it moves around towards the ventral median line. This movement takes place in common with the movement of the amnion over the body from left to right, as shown in Fig. 28. In embryo No. II, however, the liver has nearly reached the umbilicus, and the vein has almost moved around to the

¹ Kollman: His's Archiv, 1891, Plate III, Figs. 2, 3, 4. V. umbil.

ventral median line, as shown both in the reconstruction and the sections (Figs. 30, 37-39). After the vein has moved around the body to its ventral surface, and after the liver moves away from the umbilicus up to the permanent diaphragm, it is easy to explain the formation of the round and broad liga-

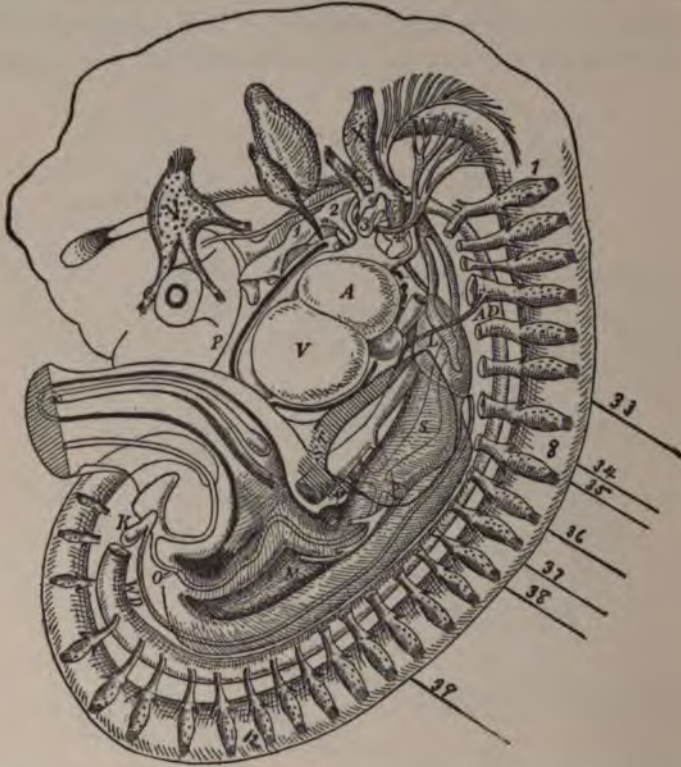


FIG. 30. — Reconstruction of Embryo No. II. Enlarged 17 times. *V* and *X*, fifth and tenth cranial nerves; *1*, *2*, *3*, and *4*, cast of the branchial pockets; *1* and *8*, first and eighth cervical nerves, from the fourth the phrenic arises; *12*, twelfth dorsal nerve; *A*, auricle; *V*, ventricle; *L*, lung; *S*, stomach; *P*, pancreas; *W D*, Wolffian body; *K*, kidney; *M*, mesentery; *S T*, septum transversum; *O*, openings which communicate with the peritoneal cavity of the opposite side. The black line around the heart marks the pericardial cavity.

ments of the liver as a secondary formation, but not as a remnant of a ventral mesentery. It might be called a portion of the septum transversum, as it is directly continuous with it. A ventral mesentery does exist between the abdominal walls and the liver, and only extends slightly below the liver. It is

always slightly to the left of the median line, and is in direct connection with the septum transversum (Fig. 30, *O* and *ST*).

Coelom of Embryo No. II. — After the body cavity is beginning to separate from the extraembryonic coelom, the next important stage is the one after the separation is complete, as from now on the adult body cavities are formed by a simple division and expansion of the cavities already within the body. This stage is represented in embryos XVIII, II, and IV. All of these embryos are nearly of the same size, the successive stages being in the order they are given. No. XVIII is somewhat distorted in the middle of the body, while No. IV is slightly macerated. No. II is a perfect specimen, and has been already described by me several years ago.¹ I shall confine my description of it to the body cavity.

The external form of the embryo within the ovum is given in Fig. 29. The position of the umbilical vesicle, as well as the extent of the amnion and the relation of the umbilical vesicle and amnion to the chorion, are all given. The umbilical cord is large and lies on the left side of the body, while in most embryos already published it is upon the right side. The cord is short, and midway between the embryo and its attachment to the chorion it shows a decided enlargement. The umbilical vesicle is large, measuring 5×7 mm., and is located between the head end of the embryo and the chorion.

The amnion has not grown very much, still leaving a great



FIG. 31. — Cast of the Body Cavity of Embryo No. II. Enlarged 22 times. *A*, position of the aorta; *V*, position of the vein; *M*, position of the mesentery; *WB*, position of Wolfian body; *P*, pericardial cavity; *L*, coelom over liver.

¹ Mall: Journ. of Morph., vol. V.

space between it and the chorion, the extraembryonic coelom (compare with Fig. 26). Within it hangs this large umbilical vesicle, the lumen of which no longer connects with the alimentary canal. The separation is now complete. Around the stem of the vesicle the extraembryonic coelom communicates freely with the body cavity, as shown in Fig. 30. This figure is from a reconstruction, and shows the general extent of the body cavity within the embryo. It encircles the heart, and then extends to the lungs and over them and to the stomach, over the intestines, and out into the cord. A cast of the



FIG. 32. — Outline of Coelom in Embryo No. II in Sagittal Section. The striated line indicates that the coelom crosses the median line. *P*, pericardial space; *pl*, pleural cavity; *lp*, outline of lesser peritoneal cavity.

whole cavity is also given, showing the slit on the dorsal side for the mesentery of the intestine, and the grooves on either side of this for the Wolffian bodies. There are also grooves in the cast for the veins, and the place where the Cuvierian duct enters the heart is marked *V*. The sagittal section of the peritoneal cavity is given in Fig. 32. The striated line indicates where the cavity crosses the median line of the body, while the other lines outline the cavity beyond. *lp* outlines the lesser peritoneal cavity. Figs. 33-39 give the extent of the peritoneal cavity in different portions of the embryo, as indicated by the lines in Fig. 30.

It is not difficult now to imagine the body cavity of embryo XII converted into the one just described. In that embryo the heart is high in the neck on the oval and dorsal side of the septum transversum. In this embryo it is on the ventral and oral side of the septum transversum, but still above the eighth cervical nerve. The septum transversum has already received its nerve supply from the fourth cervical nerve, as pointed out in the early part of the century by von Bear. This movement of the septum transversum is accompanied by a movement of all the other organs on their way into the thorax and abdomen of the future individual. In the rotation the Cuvierian duct acts much as the fixed point about which the coelom is bent.

The figures all illustrate this beautifully. But as the heart has rolled over the liver, and the septum transversum has undergone a quarter-revolution, the Cuvierian ducts and all have moved away from the head. This is by no means the end of the excursion of the septum transversum, as its dorsal end must move down and beyond the twelfth dorsal segment (compare Fig. 30).

The pericardial cavity surrounds the whole heart, as the various figures show. The cavity is traversed only where the large veins enter, and where the aorta leaves the heart. The

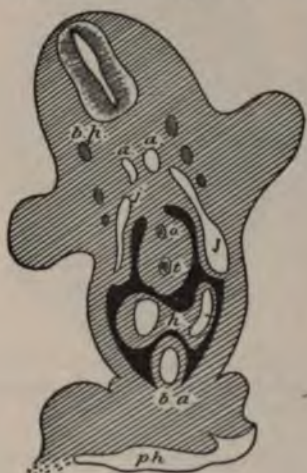


FIG. 33.



FIG. 34.

FIGS. 33 and 34.—Sections through Embryo No. II at the Points indicated in Fig. 30. Enlarged 22 times. *B h*, brachial plexus; *a*, aorta; *b a*, bulbus aortae; *p h*, pharynx; *h*, heart; *l*, trachea; *e*, oesophagus; *j*, jugular vein; *l*, lung; *v c*, cardinal vein; *C*, Cuvierian duct.

cavity completely surrounds the bulbus aortae to its origin (Figs. 32-35) in the ventricle. On the dorsal side of the heart the pericardial cavity is separated by a bridge for the transmission of the veins to the heart. Between the bulbus aortae and the entrance of the veins into the heart the pericardial cavity crosses the median line as three distinct openings, as expressed by the black areas in front of the trachea in Fig. 30. On the dorsal side of the heart on either side of the lungs the pericardial cavity communicates with the pleural cavities by means of two openings (Fig. 33), each of which is about .1 X .5 mm. in diameter. Farther on, the pleural cavities

extend as two slits which encircle the lobes of the liver and separate them from the alimentary canal on the one hand and from the body wall on the other (Figs. 34-37). The two

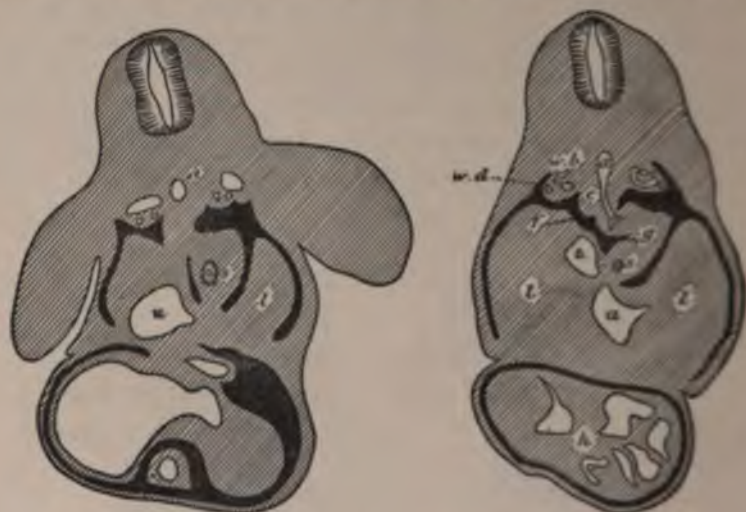


FIG. 35.

FIG. 36.

FIGS. 35 and 36. — Sections through Embryo No. 11. *A*, aorta; *s*, stomach; *l*, liver; *u*, umbilical vein; *x*, bulbus aortae; *h*, heart; *e*, omphalomesenteric vein; *g*, lesser peritoneal cavity; *f*, foramen of Winslow; *c*, coeliac axis; *w* *h*, Wolffian body; *w* *d*, Wolffian duct.

pleural cavities do not communicate with each other around the lungs, leaving for them both a dorsal and a ventral mesentery.

This appearance of the coelom about the lungs and the liver can be explained by the lungs and liver both growing into the two pleural cavities of embryo XII, and this has often made me think that the membrana reuniens of embryo XII is the main origin of what is called septum transversum in embryo II. If this proves to be the case, then the lower end of the membrana reuniens will form the ventral end of the diaphragm, and not the reverse. A stage between embryos XII and XVIII (Fig. 41) is required to elucidate this point.

In the neighborhood of the stomach the peritoneal cavity on either side of it has become asymmetrical, as Fig. 36 shows. The mesentery has become bent to the left side, leaving a diverticulum from the right side which extends oralwards to the tip of the lung (Figs. 34 and 35) to form the beginning of

the lesser peritoneal cavity.¹ Further aboralwards the cavities become symmetrical again (Figs. 37, 38), and then unite along the ventral median line, as shown in Fig. 39. The ventral mesentery shown in Fig. 38 does not extend more than a section or two beyond the liver, and is separated by a marked



FIG. 37.



FIG. 38.



FIG. 39.

FIGS. 37-39. — Sections through Embryo No. II. *A*, aorta; *v.c*, cardinal vein; *o*, omphalomesenteric vein; *p*, pancreas; *i*, intestine; *b*, bile duct; *l*, liver; *h*, heart; *u*, umbilical vein; *m*, mesentery; *w.b*, Wolffian body; *all*, allantois.

opening from the stem of the umbilical vesicle in this embryo, as is shown in Fig. 30, *O* (see also No. XII, Fig. 16, *O*). On the aboral side of the umbilical cord the peritoneal cavities of the two sides unite in both embryos again, marked *O'* in both figures.

Development of Body Cavity in the Chick.—The body cavity of the chick has been carefully studied by Budge,² who followed its course by means of injection. With a fine hypodermic syringe he filled the spaces forming the coelom in the order of their appearance, thus showing their extent in various embryos. The splanchnopleure, according to Budge, may be split into two layers, one dorsal or lymphatic and the other ventral or vascular. Drasch's³ recent description of the early

¹ Mall: *Journ. of Morph.*, vol. V.

² Budge: *His's Archiv*, 1880 and 1887.

³ Drasch: *Anatom. Anz.*, Bd. 9.

formation of the coelom confirms this statement. As the first blood-vessels are formed, lymph vessels appear on their dorsal side, which flow together to form a network, and accompany the primitive veins to the axial part of the germinal area. Here the lymphatics form two spaces, one on either side of the body, which soon unite across the body on the ventral side of the heart. In this way the primitive body cavity of birds appears at first as an H, the uprights of which are on either side of the body and the cross-piece on the oral side of the sinus venosus. In its further development the sinus venosus grows to the dorsal side of the cross-piece, thus reversing the relation of the vascular system to the coelom in this portion of the embryo. The uprights of the H fall to the outside of the body, and are swallowed up in the formation of the amniotic folds.

According to Budge two diverticula grow from the cross-piece of the H, one on either side of the chorda, towards the



FIG. 40. — Section of a Chick to show that the Body Cavity communicates with the Extraembryonic Coelom. Although the embryo has been injected, the injection masses *a* and *c* are not continuous.

tail of the embryo, to form the primitive pleuro-peritoneal cavities. Budge's paper was published from fragmentary notes after his death, and I am sure that the above statement is not correct. Professor His has placed at my disposal Budge's specimens, which I think show conclusively that his interpretation

of this subject is not correct. Most of the injections were made into the amniotic fold, which is very large in birds. Cross-sections of chicks at this stage show that the large extraembryonic coelom communicates very freely with the body cavity, and the cross-piece also communicates freely with the cavity at the anterior end of the embryo. This has already been described and pictured by Cadiat,¹ and recently again by Duval.² Around the heart, however, the communication is freest between the extraembryonic coelom and the body cavity, and it is natural

¹ Cadiat: Jour. de l'Anat. et de la Physiol., 1883, Plate V, Figs. 1 and 2.

² Duval: Atlas d'Embryologie, Plate XXII, Fig. 354.

that the fluid should find its way along this channel first, and then extend into the body cavity from this point, giving pictures which in transverse section are like Fig. 40. Surface views could not decide that these cavities communicate freely; and these sections which I have studied were no doubt made after Budge had written the rough draft of his manuscript, as they are not referred to in his paper.

Although the body cavity of the bird is formed after the same manner as it is in the human embryo, there is one marked difference in the formation of the pericardial space. In the bird the mesoderm does not extend throughout the head fold of the blastoderm, leaving a portion of the ectoderm in direct contact with the entoderm. Later the mesoderm grows into this region, and at the same time the pericardial cavity extends to the outside of the body. This condition continues for a long time, allowing the pericardial cavity to communicate with the false amnion after the embryo is well formed. Duval's excellent *Atlas* shows how the pericardial cavity first communicates with the exterior of the body, and after the body walls have united the heart still lies in apposition with the liver, as there is no septum transversum. The only trace of a septum transversum that I can find in young chicks is at the point the Cuvierian ducts enter the heart. Here a bridge of tissue passes transversely to the body. The liver does not grow into it, but accompanies the single omphalomesenteric vein before it enters the heart.¹

Development of the Diaphragm.—Our knowledge of the development of the diaphragm is based upon the researches of von Baer,² Cadiat,³ His,⁴ Uskow,⁵ and Ravn.⁶ Each contributed his portion: von Baer that the diaphragm is at first located high in the neck and must descend in its development, and, because of its high position at first, is innervated by a

¹ Since the above has been written this subject has been studied thoroughly by Ravn, whose paper will be found in His's Archiv, 1896.

² Von Baer: *Entwicklungsgeschichte*, 1837.

³ Cadiat: *Jour. de l'Anat. et de la Physiol.*, 1878.

⁴ His: *Anat. mensch. Embryonen*, Th. I, 1880.

⁵ Uskow: *Arch. f. mik. Anat.*, 1883.

⁶ Ravn: *His's Archiv*, 1889.

cervical nerve; Cadiat and His recognized the mass of tissue in the embryo which is destined to give rise to the diaphragm; Uskow and Ravn studied more carefully the separation of the body cavities from one another; and the wandering of the organs was emphasized by Uskow.

It is now no great task for me to give the development of the diaphragm in the human embryo, for I have at my disposal excellent sections, as well as definite knowledge of the anatomy of the surrounding organs contributed by the above-mentioned authors.

While the embryo is still straight it is very easy to locate the various organs and their relations to one another, but through their shifting and the flexion and extension of the embryo the relations are constantly changing, and one must not rely too much upon sections, or else erroneous impressions will often be obtained. At first the heart is upon the oral and dorsal side of the septum transversum, then on its ventral side, and finally again on its dorsal side. At first the lungs are on the dorsal side of the heart, then on the lateral side, and finally also on the ventral side of it. At first the liver is on the aboral side of the septum transversum in the head of the embryo, then on the dorsal side of it in the cervical region of the embryo, then as the liver is descending in its excursion it is transferred to the ventral side of the septum and extends into the sacral region. At first the Wolffian body extends high into the thoracic region of the embryo, but while it is degenerating and the diaphragm descends, the upper part of the posterior cardinal vein remains, while the lower part is incorporated with its vena cava inferior, as shown by Hochstetter.¹ As the Cuvierian ducts and cardinal vein descend into the thorax, the segmental veins entering the cardinal veins are gradually shifted, so that veins which originally emptied into the posterior cardinal now empty into the anterior cardinal. While the whole process is taking place the arteries arising from the descending aorta also shift, as I have shown in a previous communication.² At that time my collection of human embryos was very limited, and it

¹ Hochstetter: *Morph. Jahr.*, Bd. 20, p. 563.

² Mall: *Journ. of Morph.*, vol. V, p. 472.

was necessary to include some observations on lower animals to prove my point, but now I can give a complete table of human embryos in which the point of origin of the coeliac axis is recorded.

TABLE SHOWING POINT OF ORIGIN OF COELIAC AXIS.

EMBRYO.	LENGTH IN MILLIMETERS.	ORIGIN OF COELIAC AXIS.
No. XII.	2.1	Opposite 4th cervical nerve. ¹
His's Embryo M	2.6	" 1st dorsal " ²
" " B	7.	" 2nd " " ³
No. II.	7.	" 4th " " "
His's Embryo A	7.5	" 6th " " ⁴
No. XLIII.	13.	" 10th " " "
No. IX.	14.	" 11th " " "
No. XXII.	18.	" 11th " " "
No. XVII.	16.	" 12th " " "
No. LVII.	20.	Below 12th " " "
Adult.		" 12th " " "

¹ In the first two embryos the omphalomesenteric artery is noted, and not the coeliac axis.

² Compare Fig. 15, Plate VI, His's Atlas, with M4, Pl. VII.

³ Compare Fig. 35, Plate II, His's Atlas, with Fig. 1, Plate I.

⁴ Compare Figs. 79 and 86, His's Atlas, with Fig. 4, Plate I.

The table shows that the arteries arising from the ventral side of the aorta to supply the stomach and intestines are constantly shifting until their definite origin is finally reached. In these specimens the omphalomesenteric artery is shifted ahead of the coeliac axis. In embryo No. II the omphalomesenteric artery has a double origin from the aorta, which indicates that this movement may be brought about by a new anastomosis forming, which is then followed by an occlusion of the old origin. At any rate it is impossible that the whole aorta shifts with the abdominal viscera, for it is bound to the vertebrae and muscle plates through the segmental arteries.

The various sections and the reconstruction of embryo No. II show the pleural and pericardial cavities still communicating freely. The same is true in embryos XIX, XVIII, and IV.

Immediately after this stage there are no embryos in my collection, so I have no specimen in which the communications between the pleural and pericardial cavities are just closing.

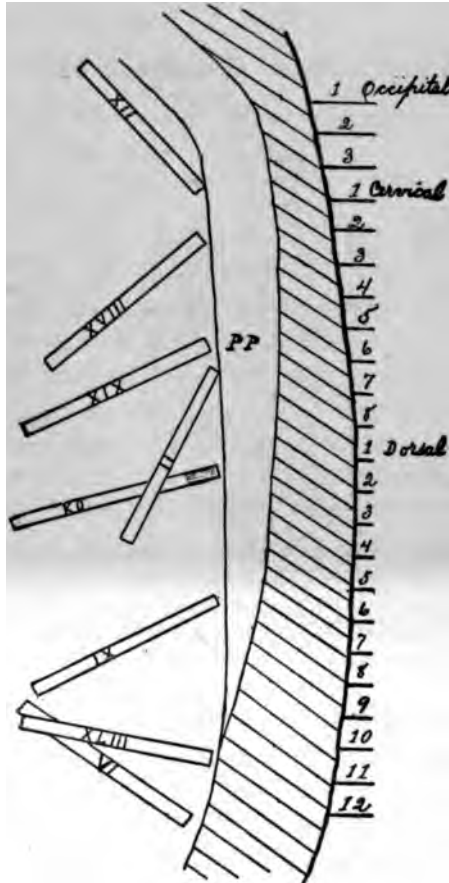


FIG. 41. — Diagram to show the Position of the Diaphragm. The numbers on the blocks indicate the embryos from which the diaphragms are taken. *KO* is His's embryo *K O*; *PP*, the outline of the opening between the pleural and peritoneal cavity, which is finally closed when the diaphragm reaches the tenth dorsal segment.

In embryos VIII, V, IX, and XLIII (Figs. 41-43), the pleural and pericardial cavities are separated, while the pleural and peritoneal still communicate. In the embryos with a vertex-breech measurement exceeding 17 mm. the pleural and peritoneal have been separated completely.

The separation of the pleural from the pericardial cavity is dependent upon the complete development of the diaphragm. At first the septum transversum and the membrana reuniens are

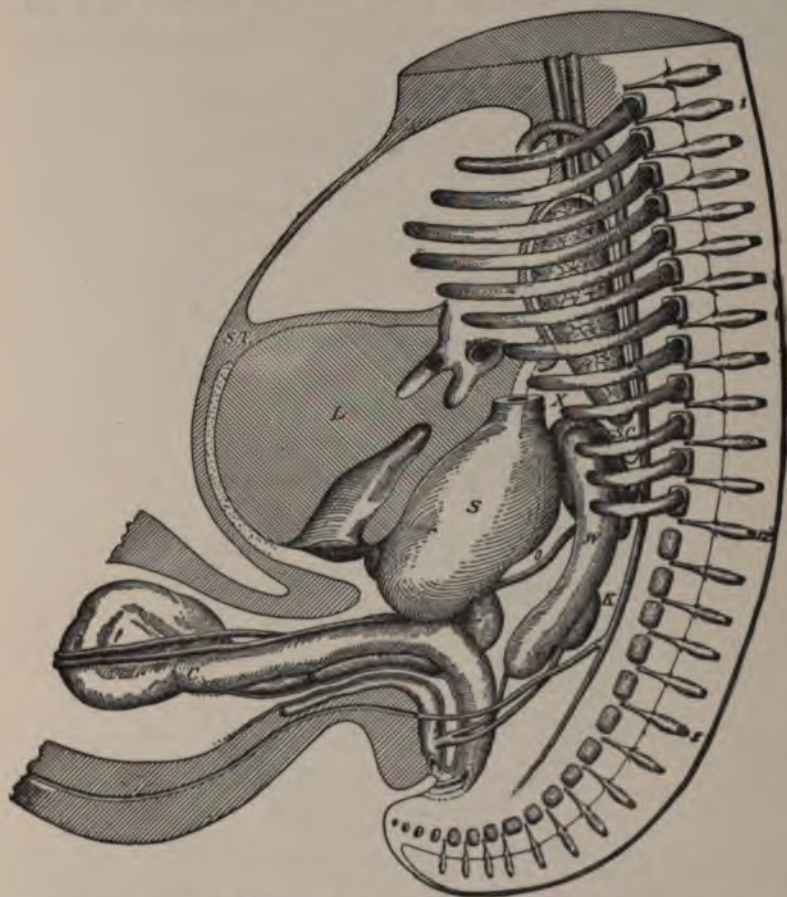


FIG. 42. — Reconstruction of Embryo No. IX. Enlarged 17 times. *ST*, septum transversum; *L*, liver; *S*, stomach; *C*, caecum; *W*, Wolffian body; *K*, kidney; *t-12*, dorsal ganglia; *e*, omphalomesenteric artery. The ventral mesentery of the liver is dotted, as it is only a thin membrane; *S C*, suprarenal capsule; *X*, point of communication between pleural and peritoneal cavities.

on the ventral side of the pleural cavity, and both are still located within the head. As the septum transversum descends into the body it is next located on the dorsal side of the heart. In other words, the dorsal end of the septum transversum has not moved as rapidly as the ventral end, and thus the whole

mass of tissue has turned a quarter revolution. This is accompanied by the extreme flexion of the head, as represented in embryo No. II. At this time the septum transversum has descended to the lower part of the cervical region. Now the septum begins to turn in the other direction again, for with the

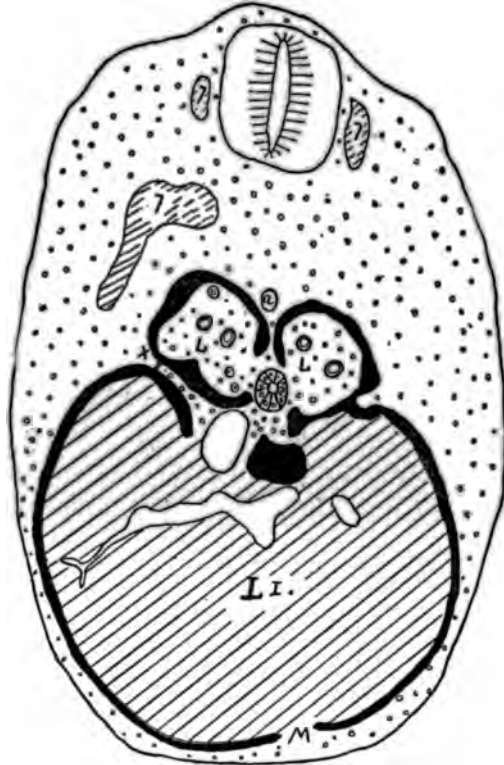


FIG. 43. — Section through the Point of Communication between the Pleural and Peritoneal Cavities in Embryo No. IX. Enlarged 15 times. 7, seventh rib; L, lung; L_i, liver; M, ventral mesentery of liver; a, aorta. The diaphragm is complete on one side, X, while it is incomplete on the other.

development of the neck the ventral end of the septum becomes the fixed point and the dorsal end moves more rapidly. The successive stages in the movement of the septum are best shown in the diagrammatic Fig. 41.

Fig. 42 shows the septum transversum on the ventral side of the stomach and the pleural cavity communicating with the peritoneal at the point X. The Wolffian body and the supra-

renal capsule, which is very large, have receded markedly, and the pleural cavity already forms a pocket on the dorsal side of them. A sagittal section through this region, somewhat

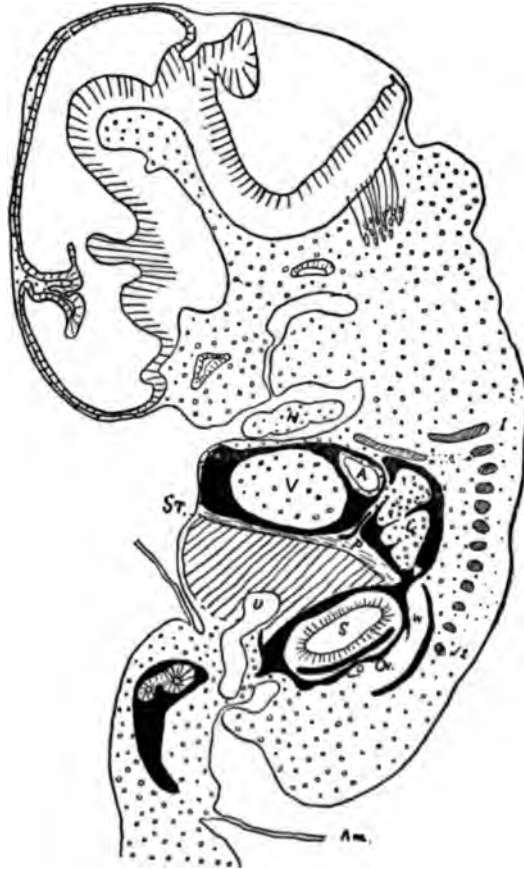


FIG. 44. — Section through Embryo No. XLIII. Enlarged 8 times. *H*, hand; *A*, auricle; *V*, ventricle; *L*, lung; *S T*, septum transversum; *P*, phrenic nerve; *U*, umbilical vein; *S*, stomach; *W*, Wolffian body; *Ov*, ovary; *Am*, amnion; *r-rs*, ribs.

distant from the median line, is given in Fig. 44. A transverse section of the embryo pictured in Fig. 42 is given in Fig. 43. This section is just at the point above the opening, and shows the communication between its pleural and peritoneal cavities closed on one side, but open on the other. There is a ridge on the side of the cavity which projects between the lung and

the liver and continues down to the suprarenal capsule. This ridge has been well described by Ravn,¹ who gives an excellent illustration of the opening with the ridge encircling it.

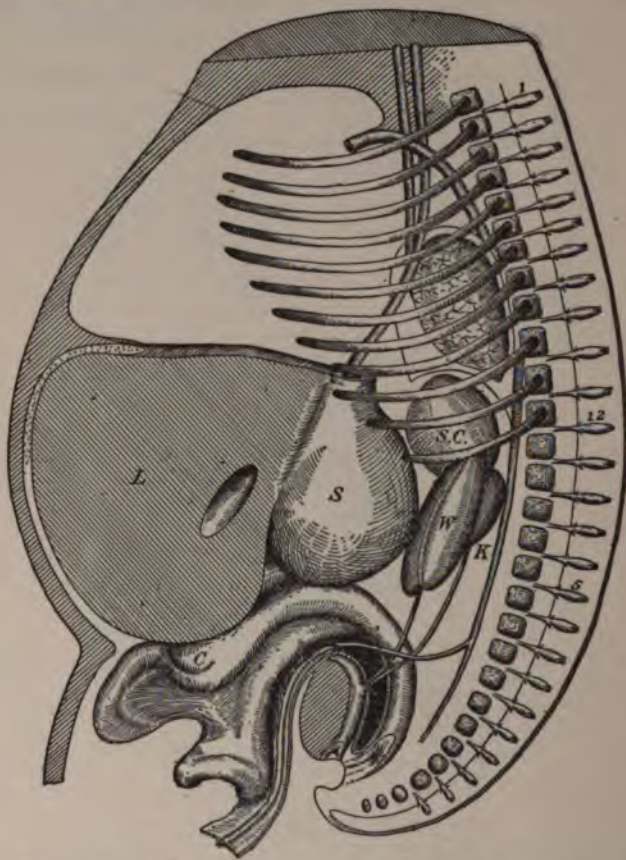


FIG. 45.—Reconstruction of Embryo No. X. Enlarged 8 times. 1-12, dorsal ganglia; S C, suprarenal capsule; W, Wolffian body; K, kidney; L, liver; S, stomach; C, caecum. The dotted area on the ventral side of the liver indicates the extent of the ventral mesentery of the liver.

In all the embryos in which the pleural and peritoneal cavities still communicate, the vena cava does not yet communicate with the posterior cardinal vein.

Fig. 45 is from an embryo slightly larger than the one from which Fig. 42 was taken. The pleuro-peritoneal communica-

¹ Ravn: His's Archiv, 1889, Plate X, Fig. 16.

tion has just closed by the walls of the ridge having grown together; the extent and shape of the pleural cavity is much as it is in Fig. 42. The Wolffian body is smaller, and the kidney and suprarenal capsule have come together.

The story, then, is brief: as the diaphragm descends, its dorsal end is in apposition with the suprarenal capsule, and finally, when the capsule approaches the twelfth rib, a ridge of tissue which also includes the capsule unites with a ridge from the septum transversum, and the opening is closed. These two ridges, however, are portions of one and the same ridge, as they form a circle and in section appear as two ridges. The circle is closed much after the fashion of tying up a bag.

All of the abdominal organs, with the exception of the kidney, descend; and the descent is not completed until the pelvis is formed to admit some of them. In the stages pictured nearly all the small intestine lies in the umbilical cord, as is the case in many mammalian embryos. In embryo X (Fig. 45) a large portion of the liver also projects into the cord. I have also observed a hernia of the liver in another embryo somewhat larger. I do not consider the form of embryo X altogether normal, but this was not noticed until the reconstruction was complete.

Closely associated with the closing of the pleuro-peritoneal opening is the development of the coeliac ganglion. In these young embryos it is extremely large, and can be outlined already, while the septum transversum is still high in the thorax. As the septum descends, the various communicating branches of the nerves are caught up with the coeliac ganglion and dragged along. This accounts for the high origin of the splanchnic nerve.

Fig. 46 (embryo VI) shows that all the tissues are becoming more definitely outlined, and the whole structure is firmer than in embryo X. The organs of the abdomen are more firmly clustered together, and the intestine has become more convoluted. The lung is much larger, and the pleural cavity extends to the ventral wall of the embryo, obscuring wholly the outline of the heart. In general it confirms everything given in Fig. 45.

Minot¹ has stated that the pleural cavities are to be considered a portion of the septum transversum, because they lie on the dorsal side of it. From what has already been said above it will be seen that I consider the septum transversum the

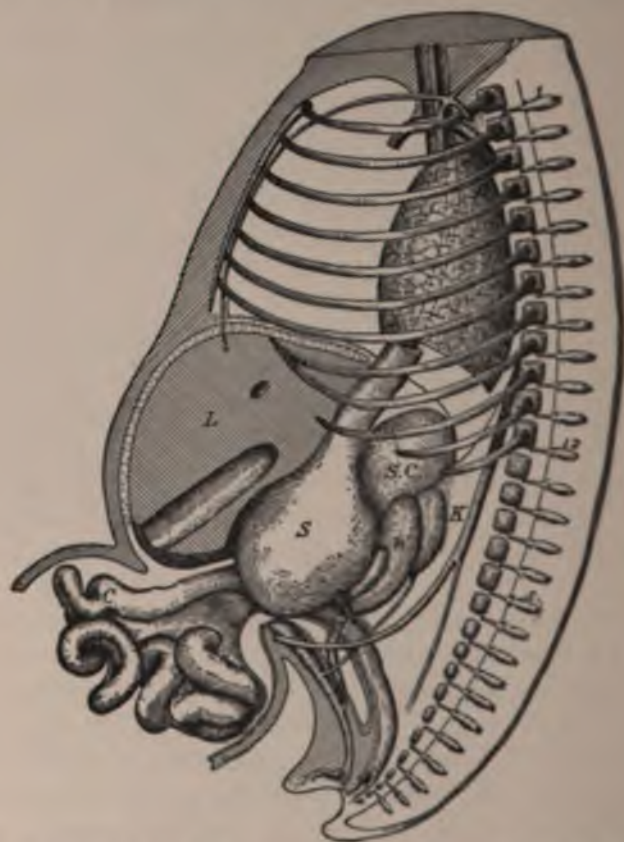


FIG. 46. — Reconstruction of Embryo No. VI. Enlarged 8 times. *1-12*, dorsal ganglia; *S.C.*, supra-renal capsule; *K*, kidney; *W*, Wolffian body; *S*, stomach; *C*, caecum; *L*, liver. The dorsal area on the ventral side of the liver indicates the extent of the ventral mesentery.

mass of tissue between the pericardial cavity, the pleural cavities, and the opening between the two sides of the peritoneal cavity immediately below the liver, marked *O* in Figs. 16 and 30. This tissue includes the membrana reuniens, which is really the wings of the septum transversum as described by His. In my

¹ Minot: Human Embryology, p. 483.

account I have employed the term *membrana reuniens* wherever possible to avoid confusion, and have usually employed the terms *septum* and *primitive diaphragm* as synonyms.

There are developed within the region of the *septum transversum* the whole liver, including its ventral mesentery, the lesser peritoneal cavity, the stomach, and the suprarenal capsule. This same region which I have marked out by these three boundaries as the *septum transversum* is still sharply defined in the adult. The point *O* in Figs. 16 and 30 is still as definably marked as ever by the round ligament, foramen of Winslow, and the duct passing from the liver to the duodenum. The round ligament is developed by the umbilical vein shifting around the side of the abdominal walls into the ventral mesentery of the liver, and then when the liver is retracted from the umbilical cord, the vein and mesentery remain as the round and broad ligaments respectively.

Lesser Peritoneal Cavity.—I have already discussed the lesser peritoneal cavity in a separate paper,¹ and find that I can confirm all that I have stated at that time. I can only add that the portion of it extending up under the lung degenerates, while the omental sac is growing rapidly. I have also found that it is extremely easy for the omentum to find its way over the large intestine. At the time this takes place the large intestine is in the median line, while the stomach and the omentum are on the left side of the body. After the intestine is retracted from the cord the caecum falls over to the right side of the body, while the descending colon is shifted to the left side, and the omentum then comes to lie on the ventral side of the transverse colon.

Expansion of the Body Cavity and Obliteration of the Extra-embryonic Coelom.—After the pleural and pericardial cavities are separated from each other it is very easy to follow their further development. In embryo II, Fig. 47, the heart is still upright, and a transverse section of it is also transverse to the lung. The pleural cavity lies wholly on the dorsal side of the pericardial, Fig. 32. In the next stage, as the lungs descend more and more, the heart is tilted over so that its base is towards the

¹ Mall: *Journ. of Morph.*, vol. V.

lung and its apex away from it, as in embryo IX, shown in Figs. 42 and 48. The pericardial space has now become separated completely from the pleural, although both have grown at about the same pace. From now on the pleural space grows more rapidly than the pericardial, as shown in Fig. 49. I have a number of embryos which represent intermediate stages between embryos IX and XXII, and all of them confirm the idea that the pleural space develops first and then is followed by a growth of the lung. Fig. 50, which is a section of embryo No. XLV, shows a marked increase in the size of the lung, but the heart and pericardial space are of about the same

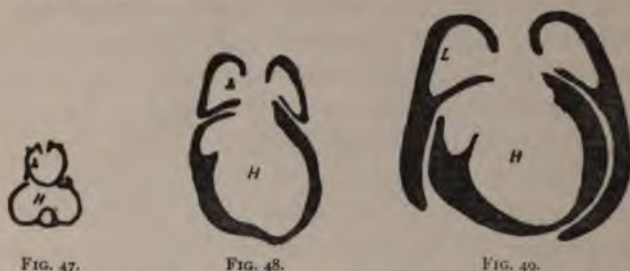


FIG. 47-49. — Outlines of the Pleural and Pericardial Cavities to show their Relative Position and Size. Enlarged 7 times. Fig. 47, Embryo No. II; Fig. 48, Embryo No. IX; Fig. 49, Embryo No. XXII. *H*, position of heart; *L*, position of lung.

size as in embryo XXII. A much later stage is shown in Fig. 51. The scale of enlargement is only half that of Fig. 50, and when this is considered it is again seen that the heart has not grown very much but the lung has developed enormously.

It is therefore seen that at first the pericardial cavity is on the oral side of the pleural, then on the ventral side, and is finally enclosed by the pleural cavity growing over it.

The growth of the pleural cavity over the pericardial accounts for the location of the phrenic nerve in the adult. In Fig. 47 the nerve passes to the septum transversum from the lateral body wall and it is gradually separated from it by the descent of the septum and by the growth of the pleural cavity between the nerve and the body wall, thus locating the nerve in a membrane, as Figs. 48 and 49 will readily explain.

The expansion of the peritoneal cavity is by no means as simple. In it there are many bands and mesenteries, as

well as a marked shifting of the organs. With the descent of the testis a portion of it is cut off to form the tunica vaginalis.

In embryo II the peritoneal cavity is extremely simple, as the figures show, — a simple cavity on each side, communicating the one with the other by means of two openings, one above and one below the omphalomesenteric duct. Later, as the diaphragm descends more and more, the liver rotates, and its lobes soon fill the peritoneal cavity, while the intestine develops out into the cord. The Wolffian body, sexual glands, and suprarenal capsule fill the dorsal side of the cavity and the rudimentary pelvis. The whole development of the intestine takes place within the cord, and finally it is drawn into the embryos when it is about 30 mm. in length. By what process this takes place I am unable to determine, but it must take place very rapidly, for I have never seen a human embryo in which it is only partly retracted. In the pig's embryo, however, I have found the stages in which the intestine is in process of retraction.

The liver now fills nearly the whole cavity, and extends down to the pelvis, and in embryo XXII projects over the ovary and is in contact with the rectum. As the intestines are retracted from the cord the liver is relatively higher and higher, for the expansion of the abdominal walls is now greater below the umbilical cord than before, giving more space in this region for the intestine which displaces the liver. In embryos XXXIV and XLVIII the intestines have been studied, and it was found that they were still located in the ventral portion of the peritoneal cavity, as there is no pelvic cavity large enough



FIG. 50. — Outline of Pleural and Pericardial Cavities in Embryo No. XLV. Enlarged 7 times.



FIG. 51. — Outline of Pleural and Pericardial Cavities in Embryo No. XXXIV. Enlarged $3\frac{1}{2}$ times. H, position of heart; L, position of lung.

to hold any portion of them. This question will be discussed in the next paper.

The extraembryonic coelom has only a short existence, as it is already completely obliterated in embryo No. XXII. This embryo came to me in an unopened ovum, and on this account



FIG. 52. — Embryo No. XXII within the Ovum. Enlarged 3 diameters. The villi of chorion are outlined on one side of the ovum only. The umbilical vesicle, *u. v.*, has become shifted around to the dorsal and right side of the embryo. The outline is made from a photograph, and is correct in detail with the exception of the attachment of the chord to the chorion. This in reality attaches itself to the chorion immediately to the right of the embryo.

is extremely valuable for this purpose. This embryo is about six weeks old, so, reasoning from it, the union of the amnion and chorion takes place earlier than is generally believed. In embryo No. XLIII, which is about five weeks old, the amnion has expanded over the whole embryo and has nearly reached the chorion. The earlier stages are given in the sagittal sections. They show that the amnion is nearest the chorion at the caudal end of the embryo in the earliest stages, and soon

the two unite at this point. As the embryo grows, the union of amnion and chorion extends. At the end of the fourth week the extraembryonic coelom is still very large ; at the end of the fifth week the space between the embryo and chorion is divided equally between the amnion cavity and the coelom ; at the end of six weeks the extraembryonic coelom has disappeared.

BALTIMORE, May 5, 1896.

A CONTRIBUTION TO THE STUDY OF VARIATION.

(SKELETAL VARIATIONS OF *NECTURUS MACULATUS* R.A.F.)

HERMON C. BUMPUS.

"Both heredity and variation are in urgent need of causal explanation."—Roux, Wheeler.

In this communication, based upon the comparative examination of skeletons of *Necturus*, an effort is made to answer the following questions:

I. What is the per cent of homœotic variation¹ in the attachment of the pelvic arch to the axial skeleton; is there true meristic variation, and is homœotic associated with meristic variation?

II. Is there a ratio between the absolute length of the animal and the number of vertebræ?

III. Why does the variation tend towards forward rather than backward homœosis?

IV. Can an explanation be given for the frequent occurrence of oblique or unsymmetrical sacra?

V. Is the position of the pelvic arch dependent upon the ordinal position of some one segment (sacrum) of the vertebral column, or is its position determined by the location of some topographical point?

VI. Are there variations in the position of the pectoral arch, and are these correlated with variations in the pelvic arch?

VII. Are there other skeletal variations associated with pelvic variation?

¹ Bateson ('94) states that "Homœotic variation in the spinal column consists in the assumption by one or more vertebræ of a structure which in the type is proper to vertebræ in a different ordinal position in the series. Examples of this are seen in the . . . occurrence of a vertebra, normally lumbar, in the likeness of a sacral vertebra, having its transverse process modified to support the pelvic girdle. . . . In using the expression, Homœosis, . . . we may speak of the variation as occurring from before backwards or from behind forwards, according as the segment to whose form an approach is made stands in the normal series behind or in front of the segment whose variation is being considered. The formation of a cervical rib on the VII vertebra is thus a backward Homœosis for the VII vertebra thus makes an approach to the characters of the VIII," etc.

VIII. Are variations more frequent in males than in females?

IX. Are there anatomical grounds for the theory of vertebral intercalation?

METHODS.

Until within a few months the examination of a large series of skeletons involved the expenditure of considerable time for their proper preparation, the destruction of valuable anatomical material, the occasional loss of certain cartilages, and the too frequent misplacement of disjointed parts. The truly wonderful discovery of Röntgen, however, has placed in the hands of the anatomist an economical means for the most critical examination of the bones, *in situ*, without the use of macerating fluids or the scalpel, and a means readily applicable, without injury to the most valuable museum specimen or even to the living animal.

The one hundred alcoholic specimens on the comparative examination of which this paper is based, belong to the museums of Brown University and the Boston Society of Natural History. Each animal, properly numbered, was bound in a proper position to a thin piece of board, and the board with the specimen attached was then placed upon an envelope of black paper containing a common photographic plate. When large plates were used as many as ten animals were exposed at the same time.

Immediately over the table on which lay the photographic plate, and approximately 500 mm. distant, was suspended a Crooke's tube of the Elihu Thomson pattern, rendered fluorescent by means of a Tesla coil. The exposure was invariably five minutes. When the tube became dim it was warmed with a Bunsen flame until the return of the brighter illumination. The plates were developed with the ordinary "pyro" developer.

The negatives, taken so as to show both the dorsal and lateral views of the vertebral column, were so clear that the ultimate joints of the tail could be readily counted. The amphicœlous structure of the vertebræ and the minute pores of the bones were often exhibited with remarkable detail. A

few specimens showed the opaque fish-hook, and thus betrayed the mode of their capture.

SECTION I.

(1) What is the per cent of homœotic variation in the attachment of the pelvic arch to the axial skeleton?

This question was considered by G. H. Parker ('96), who based his conclusions on an examination of twenty-seven specimens, cleaned by a preparator, in none of which could the total number of vertebræ be determined. Of these specimens two were found with oblique or unsymmetrical sacra. In nineteen cases the sacrum was developed from the XIX vertebra and in six from the XX vertebra. Arranged in the form of a per cent table, Parker's results were as follows :

The pelvis is attached to the XIX vertebra in 70% of 27 specimens.									
"	"	"	"	"	"	XX	"	"	22% " " "
"	"	"	"	obliquely			"	"	7% " " "
"	"	"	"	abnormally			"	"	29% " " "

An examination of a larger number of specimens shows that the number of variable individuals may be considerably increased. On Plate C the pelvic appendages are represented by short lines crossing the vertical ordinates on the abscissas of the XVIII, XIX, and XX vertebra.

The pelvis is attached to the XIX vertebra in 64% of 100 specimens.									
"	"	"	"	"	"	XX	"	"	28% " " "
"	"	"	"	obliquely			"	"	8% " " "
"	"	"	"	abnormally			"	"	36% " " "

The pelvis, then, is attached abnormally in 36% (28 + 8) of the 100 specimens. This variation should, in the light of the 127 specimens thus far tabulated by Parker and myself, be corrected by uniting the two sets of figures. This union gives the remarkable variation formulated in the following table, *vis.*, an average of 35%, and offers an example of excessive variation in animals not domestic.

The pelvis is attached to the XIX vertebra in 65% of 127 specimens.									
"	"	"	"	"	"	XX	"	"	27% " " "
"	"	"	"	obliquely			"	"	8% " " "
"	"	"	"	abnormally			"	"	35% " " "

(2) Is there true meristic variation?

Bateson ('94, p. 102) states that "numerical change may be brought about in the series of vertebræ by two different processes: first, by variation in the total number of segments comprising the whole column, in which case the variation is truly meristic; and, secondly, by variation in the number or ordinal position of the vertebræ comprised in one or more regions of the column, not necessarily involving change in the total number of segments forming the whole series, and in this case the variation is homœotic." He further states that though the latter form of variation may be associated with the former it is rarely possible in any particular case "to distinguish clearly whether such a change has occurred or not," because the terminal joints of the caudal vertebræ cannot be readily enumerated.

The application of the Röntgen rays often so clearly defines the centers of ossification of even the terminal caudal vertebræ that I have attempted to plot the several series on Plate C, though in a few cases, due to the imperfection of the specimens, tails in process of regeneration being quite frequent among the larger individuals, the number of the last few vertebræ have not been determined with certainty. Such specimens, five in number, are designated with ? over the *estimated* terminal joint.

An examination of the plotted curve of the vertebral columns on Plate C will reveal an extremely irregular line passing from Specimen 1, with 45 vertebræ, to 2 with 44, 3 with 47, etc. (Plate B, Specs. 1 and 2). There is, then, considerable meristic variation, and the second of the questions considered in this section is answered in the affirmative. Since it is possible to enumerate the caudal vertebræ, we are in a position to answer the third question, *viz.*:

(3) Is homœotic associated with meristic variation?

On Plate C the dotted ordinates indicate the specimens which are homœotic, and, taking the specimens in blocks of twenty, let us see if the homœotic specimens of each block have a greater variation in the number of vertebræ than do the normal specimens.

In the first block of twenty specimens the average number of vertebræ for each animal is 45.7. The sum of the departures from this mean in sixteen normal specimens is 22.4, giving in this block an average departure from the mean, for each specimen having the pelvis on the XIX vertebra, of 1.4. The four homœotic specimens, however, show an average departure of 1.6, *i.e.*, the amplitude of variation among the homœotic specimens is greater. The second, third, and fourth groups of twenty also reveal greater amplitude of variation on the part of homœotic individuals, though the last group is exceptional, the homœotic specimens showing less variation than the normal. The tabulated results are as follows:

The mean amplitude of meristic variation of the normal specimens

In the first 20	is	1.4	and of the homœotic specimens is	1.60.
In the second 20	"	.94	" " " "	" " 1.97.
In the third 20	"	1.35	" " " "	" " 1.50.
In the fourth 20	"	1.52	" " " "	" " 1.81.
In the fifth 20	"	1.66	" " " "	" " 1.19.
In 100	"	1.37	" " all the "	" " 1.61.

Though in this table there is considerable irregularity in the ratios, it seems to the writer that the final figures, 1.37 and 1.61, are sufficient to warrant the conclusion that specimens having abnormally placed sacra (homœotic) do present a considerably increased meristic variation, and aside from the fact that there are twice as many homœotic specimens on the right as on the left of Plate C.

Is the converse true? Do specimens presenting extremes in meristic vertebral variation tend towards homœosis?

An examination of the facts in the case brings out most striking results. There are only two specimens whose vertebræ are reduced to the abnormal number of forty-three, and both of these are homœotic. Of the nine examples bearing forty-four vertebræ, only 33% are homœotic. Of the twenty-one specimens bearing forty-five vertebræ, only 19% are homœotic. As the vertebræ now leave the normal and tend towards the other extreme, homœosis becomes more frequent, as will be observed by reference to the table.

Of 2 specimens with 43 vertebrae, 100% are homœotic.

" 9	"	" 44	"	33%	"	"
" 21	"	" 45	"	19%	"	"
" 18	"	" 46	"	29%	"	"
" 14	"	" 47	"	36%	"	"
" 16	"	" 48	"	44%	"	"
" 11	"	" 49	"	45%	"	"
" 5	"	" 50	"	60%	"	"
" 4	"	" 51	"	50%	"	"

A profile curve, or "curve of frequency" (Galton, '89), illustrating the relative distribution of the several columns according to their vertebral enumeration, is drawn in dots at the right of Plate C, the length of the ordinates being determined by the number of the specimens having respectively 43, 44, 45, 46, 47, 48, 49, 50, and 51 vertebrae. If now the respective per cents of homœotic individuals in each series be represented by a red curve on the same ordinates, we will observe that as the first curve ascends to its culminating point the second curve descends, the ordinate 45 bearing the highest point of the first curve and the lowest point of the second. To the right of this ordinate the curves converge in approximately the same way as they primarily diverged.¹

The conclusion that specimens presenting extremes of meristic vertebral variation tend towards homœosis is irresistible, and the third question of the first section is answered in the affirmative.

SECTION II.

Is there a ratio between the absolute length of the animal and the number of the vertebrae?

As before mentioned, the animals are arranged on Plate C, the smaller toward the left and the larger toward the right. The curve of relative lengths, drawn in red, passes from the specimen No. 1 at the left, with a length of only 193 mm.,

¹ The small dotted curve in red is drawn on the following scale. On abscissa 43 a dot is placed ten squares above the line which forms the right margin, and these ten squares are taken as representing the 100% of homœotic individuals having 43 vertebrae. The curve then follows the respective altitudes of 33%, 19%, 29%, 36%, 44%, 45%, 60%, and 50% on the abscissas of 44, 45, 46, 47, 48, 49, 50, and 51. I regret that the print does not make the course of this curve perfectly clear.

toward the larger specimens at the right, and of course with a constantly increasing altitude.¹ It will now be profitable for us to draw upon Plate C a line which shall represent the mean of the very irregular curve of length in terms of vertebræ. If the specimens having a larger number of vertebræ are irregularly distributed, this line will lie horizontally, but if numerical vertebral increase tends to occur in larger animals, the line will tend to rise from left to right. Suppose now we take the means used in Section I for the several blocks of twenty specimens, and, using them for our ordinates, we plot the curve of mean numerical vertebral variation. We will find it to lie between the abscissas of 45 and 48 (see dotted line on Plate C). The curve shows a *regular* rise from left to right, following the flowing curve of absolute lengths in millimeters, there being an increase of about three vertebræ in the entire series. The question at the beginning of this section is answered, then, in the affirmative.

But the simple addition of three vertebræ, each less than one millimeter in length, at the caudal end of the animal, cannot account for the increase of over one hundred millimeters in the total length of the larger specimens. The increase in length is not then to be explained by the addition of new segments behind, after the method of growth of developing arthropods and worms, but is interstitial, the individual vertebræ increasing in length with age ; though of course it does not follow that length is an absolute criterion of age. Under varying conditions growth may be more or may be less rapid.

Whether the slightly increased number of vertebræ is due to multiplication of vertebræ after sexual maturity, or is an index of unusual embryonic vitality, or is predetermined in the egg, I will not now attempt to answer, though the application of the X-rays to the developing young will definitely settle the first of these, *viz.*, the question of vertebral multiplication after sexual maturity, and the frequent occurrence of smaller and average specimens possessed of nearly the maximum number of vertebræ might indicate early extraordinary vitality, predetermination, or both.

¹ The red figures on the right of the plate indicate millimeters.

It will be observed that the rise in the curve of relative lengths is quite abrupt until the specimens are 252 mm. long, when the ascent is gradual until the specimens are 330 mm. long, from which point to the longest specimen in the series the ascent is again rapid. The curve then shows that the larger number of specimens are between 252 and 323 mm. in length. If the lengths are examined in profile, as shown by the red curve on the right of Plate C, it will be observed that the culminating point of constancy is around that specimen which has a length of 260 mm.

As the sum total of opportunities for death are directly dependent upon the length of existence, it is not surprising that the upward trend of the curve is considerably more abrupt than the descent of senescence. I am inclined to look upon the absence of specimens measuring between 245 and 255 mm., causing the depression in the ascending curve, as accidental, though it is barely possible that the depression signifies some change in the rate of growth, consequent upon the approach of sexual maturity (Minot, '91); or it may be dependent upon the season of capture. I am also at a loss to explain the depression in the curve of descent in the neighborhood of specimens having a length of 280 mm.

Before leaving this section it should be noted that the proportion of homœotic specimens is not the same among the shorter as among the longer specimens. Of the first fifty specimens, but ten are homœotic, while of the second fifty twenty-five present cases of forward homœosis.

It has already been shown that the mere addition of a few terminal caudal vertebræ cannot alone account for the increased size of homœotic specimens. The fact, then, appears, if our specimens are not exceptional, that this homœotic departure is a character of increasing frequency among such animals as have met with success in the struggle for existence. If this is the case, the present species of *Necturus* may be considered as undergoing a process of "mutation," since the causes of transformation are acting with considerable "uniformity upon large numbers of individuals" and "in a definite manner" (Scott, '94).

SECTION III.

Why does the variation tend towards forward rather than backward homœosis?

Bateson says "the attempt to apply to numerical variations the conception of variation as an oscillation about *one* mean is not easy, difficulty arising especially in regard to the choice of a unit for the estimation of divergence; . . . to judge from the scanty indications available, it seems that in cases of numerical change, variations to numbers greater than the normal number and to numbers less than it, are not generally of equal frequency. Probably no one would expect that they should be so" ('94, p. 571).

It has already been noted that in 28% of the one hundred salamanders examined, the sacral ribs are borne on the XX vertebra, while in not a single specimen are they symmetrically borne on the XVIII. Most certainly numerical variations towards a greater number, than the normal, of pre-sacral vertebræ are more frequent than variations towards a smaller number.

The positions of the oblique or unsymmetrical sacra also indicate pre-sacral increase. In only one case among the eight examples is the territory of the XVIII vertebra invaded (Plate A, Spec. 62), and this invasion marks the only encroachment upon the domain of this vertebra in a total of 127 specimens examined by Parker and myself,—an interesting fact when it is considered that the XX vertebra, no further removed *morphologically*, presents a total of 35 invasions.

Of course the easiest way of disposing of this question is to look upon the variation as atavistic. The "ancestral type" was possessed of a larger number of pre-sacral vertebræ, and the "mud-puppy" of to-day, by its anatomical variations, kindly indicates, in approximately 35% of its representatives, the character of its quasi-progenitor.

But if 35% of the present individuals tend towards pre-sacral multiplication, *i.e.*, tend to assume ancestral characters, we must not deny to these potentially progenitorial individuals the same tendency to vary that is possessed by their young, *i.e.*, 35% of the thirty-five should have an *added* increase of the pre-sacral

region, and thus about twelve specimens should have sacral ribs on the XXI or XXII vertebra. Such a disposition of the sacral ribs does not occur, though its absence may be attributed to a presumed tendency of the parent form towards "fixity" or "specific stability."

Rejecting the theory of atavistic variation, I think the forward homöosis of the sacral vertebra may be explained on other grounds.

In the first place, the origins of the processes that bear the ribs, as will be noted by reference to Pl. A, Spec. 87, arise not from the middle, but from the posterior end of each vertebra, making it a much shorter distance from the transverse process of the XIX to the territory of the XX, than from the transverse process of the XIX to the territory of the XVIII trunk metamere. It will thus seem more probable that variations occurring in the course of ontogenetic development will fall on the side of nearer proximity; and the Anlage of the limb, compounded though it may be by contributions from several trunk segments, will depart less from its normal position when it finally falls within the territory of the XX than when it falls within the territory of the XVIII vertebral segment. This explanation rests upon the assumption that the territory of the XX metamere once invaded, the secondary processes of growth within that segment will arrange for the accommodation of the sacral rib at its normal place, even though its Anlage was first located in the anterior portion of the segment.¹

A third and possibly valid explanation is offered by anatomy alone. The XVIII, XIX, and XX segments are not, in the adult at least, of equal length. The Anlage of the limb would be obliged to vary considerably more, in a linear direction, in order to influence the XVIII than it would to influence the XX vertebral segment.

Though these two possible explanations of the more frequent occurrence of forward homöosis are here given, they are both dependent upon an assumed variation in the position of the Anlage of the appendage with respect to some one

¹ The occurrence in certain fishes of pelvic fins, which lie in front of the pectoral fins, is in this connection of especial interest.

"normal" vertebral segment, *vis.*, the XIX. We assume, moreover, that when the relative position of the appendage is once determined in the embryo of *Necturus* it is determined for life; since there is no evidence to show that there is a "migration" of the parts of the pelvic region after the first rudiments are laid down. Rosenberg ('76), however, claims a true pelvic migration for *Homo*, involving an emancipation of sacral elements for the production of coccygeal vertebræ, and Credner ('86) claims a distal shifting of the pelvic arch in the fossil amphibian *Branchiosaurus*. There are grounds, moreover, as will be shown in Section V, for the belief that the very process of local differentiation of the embryonic cells, for the final production of the appendage peripheral to the vertebral axis, occurs in a position in no respect dependent upon the position of any one vertebral segment, but dependent rather upon the general proportions of the embryo as a whole. The determination of the loci of the successive vertebræ and their early differentiation exerts no determining influence on the position of the appendages. But the Anlage of the appendage once determined, influence from it will direct the growth of proper sacral elements in the nearest vertebral segment, be it the XVIII, XIX, or XX.

It should furthermore be noted that when the Anlage of the appendage does not fall within the territory of the normal metamere (XIX), its vertebra, which has presumably been producing sacral ribs in two-thirds of the specimens since some remote geological period, does not show the slightest sign of sacral differentiation, but is exactly like the other neighboring trunk vertebræ.

SECTION IV.

Can an explanation be given for the frequent occurrence of oblique or unsymmetrical sacra?

In eight per cent of the specimens examined the sacrum is not a single vertebra, but is composed of two halves, each belonging to different metameres (Plate A, Spec. 62). The legs, moreover, in these specimens, leave the body at points not directly opposite.

Those who attempt an explanation of this phenomenon on the principle of intercalation, excalation, or of pelvic migration meet with most provoking difficulties. Why should only one lateral half of a vertebra be intercalated, giving rise to a body segment which bears a single rib on one side and two ribs on the other? Or why should the process of the formation of sacral ribs involve portions of two vertebræ rather than one, and thus produce an asymmetrical sacrum?

The difficulty is partly obviated if we admit that the differentiation of the sacral vertebra is the result of centripetal influence exerted by the growing *Anlage* of the appendage. If the first rudiments of the appendages are not laid down exactly opposite each other, and there are many reasons, as, for example, the curvature of the embryo, the pressure of neighboring eggs, etc., why their primitive positions might vary, an unsymmetrical or oblique sacrum would result.

It is a remarkable fact that of the eight unsymmetrical specimens, all but one, No. 62, have the left half of the sacrum in advance of the right, *i.e.*, the axis of the sacrum is *sinistro-dextral*.

I have been quite unable to find any valid explanation for this peculiar uniformity, unless it is based upon the curvature of the embryo. Dr. Whitman has informed me that the young of *Necturus*, as it lies upon the surface of the egg, is curved, but I have not seen the embryos or larvæ, and I do not know whether the curve is lateral or dorsal-ventral, or even if it is fairly constant in its trend. An attempt to correlate the position of the sacral axis with the slightly asymmetrical position occupied by the paired viscera has proved futile.

SECTION V.

Is the position of the pelvic arch dependent upon the ordinal position of some one segment (sacrum) of the vertebral column, or is its position determined by, and is the sacrum the resultant of, the location of some topographical point?

I do not know that an attempt has ever been made to compare the distribution of the vertebræ and the location of the

appendages with the general dimensions of the vertebrate body, though the radiographs of *Necturus*, procured by the X-rays, make the necessary process of measurement extremely simple.

For a standard of measurement I have considered the distance from the first to the XXX vertebra, no matter what the actual length of the animal may be, to be represented by 100; the XXX vertebra being selected because the terminal portions of the animals are liable to injury. I have taken the intervertebral space between the XIX and XX as the locus of a variable, and I find by making accurate measurements on the negatives that the position of this intervertebral space varies in different animals in a very appreciable way. In certain animals the trunk, the part lying anteriorly to the selected intervertebral space, is relatively longer, while in others the caudal portion is relatively longer. The distance from the XX to the XXX vertebra may in some specimens (Nos. 4, 67, 95) be only 29% of the entire measured length, while in others it may be as great as 35% (Nos. 58, 61, 74).

On Plate C these relative measurements of the several specimens are given, each ordinate representing the length from the first to the XXX vertebra, and the upper of the two black lines that cross the plate represents the fluctuating intervertebral septum between the XIX and XX vertebræ. In by far the larger number of cases the caudal region is one-third, 33%, of the length. Specimen No. 1 has a trunk slightly longer and a caudal region correspondingly shorter than the average. The trunk of No. 2 is shorter and the tail longer. No. 3 is like No. 2. No. 4 has an uncommonly long trunk and short tail, etc.

If this curve is followed across the plate, it will be noted that the first nineteen vertebræ may suffer regional expansion and contraction, with correlative contraction and expansion of the caudal region, and to such an extent as to give an amplitude of variation to the dividing line between the XIX and XX vertebræ, amounting to $\frac{6}{100}$ (35% minus 29%) of the average distance between the I and XXX vertebræ. The amplitude of variation in the column itself, absolutely independent of the limbs, is sufficient, then, to include within

itself $\frac{6}{100}$ or $\frac{1}{17}$ of the measured distance. But the vertebræ in the region of the selected intervertebral spaces are, in length, considerably less than $\frac{1}{17}$ the measured distance; so that the oscillations of the dividing line between the XIX and XX vertebræ would be such as to carry it back and forth over a distance greater than one vertebra anteriorly and one posteriorly.

If we should string a series of thirty short segments of rubber tubing upon a cord, and then, after placing a card between the XIX and XX, subject the entire series to slight pressure, we would have what might roughly correspond to the column under consideration. The card, representing the selected intervertebral space, can be forced forward or backward over a certain amplitude, according to the compressibility and elasticity of the rubber rings. Suppose, while the card is at rest, we hold two fingers of the hand in such a position as to represent the rudiments of the appendages, lying on either side of the nineteenth ring. Now when the card is forced anteriorly the fingers lie no longer opposite the XIX, but opposite the XX vertebra. A forward homœosis has taken place illustrative of what happens in a large per cent of the specimens of *Necturus*.

But do our data warrant the assumption that with the compression of the anterior vertebræ there is a concomitant variation in the position of the hind limbs? When specimens tend toward elongation of the caudal region, do the legs appear on the XX, and when the reverse obtains do they tend to arise from the XIX vertebra?

A further examination of the plate will answer in a most conclusive way.

Specimens No. 58, 61, and 74 have the greatest elongation of the posterior vertebræ, and in every one of these specimens the sacral ribs are borne by the XX vertebra.

In other words, in all the specimens presenting extreme compression of the anterior vertebræ there is concomitant variation in the position of the hind limb.

Arranged on the abscissa of $34\frac{1}{2}$ (Plate C) are thirty-two specimens, and, if our theory is to be supported, a less number

of specimens should present homœotic variation than was the case with the three just mentioned, belonging to the abscissa of 35%. A reference to the plate will show that a less number, only fifteen specimens (46%), have homœotic sacra.

A still smaller number of homœotic specimens should be found on the abscissa of 35%, and, in fact, of the forty-four specimens arranged on this line but thirteen present cases of forward homœosis, 29%.

An even greater diminution in the number of homœotic specimens should be found on the abscissa of 32%, where we count fourteen specimens, only two of which, barely 14%, are homœotic.

On the abscissa of 31% among four examples there is only one that is homœotic, and among three examples on abscissa of 30% there is also only a single homœotic individual.

There are thirty-five specimens below the mean abscissa of 33% and twenty-one specimens above. The former exhibit eighteen cases of forward homœosis, *i.e.*, 51% are homœotic; the latter exhibit only four, *i.e.*, 19%.

This effort to prove that the sacral ribs are developed as the result of centripetal influence bears directly upon the proposition of Bateson "that individuality should not be attributed to members of a series which has normally a definite number of such members," for it is shown that the normal XIX vertebra owes its specialized form to the molding influence of surrounding parts, and not to some inherited directive influence upon one particular vertebra. It shows that a "redistribution of differentiation" may and frequently does take place.

The appearance of the appendage at a definite topographical point, without respect to the location of certain segments of the neighboring axial area, is in harmony with the view of Professor Whitman ('93) that the real unity, both in development and in adult stages, is the organism as a whole, "that the organism dominates cell formation."

The view here advanced also receives direct support from the facts of embryology, as shown by the following extract from Weidersheim's "Grundriss" ('93): "Mit anderen Worten — und ganz dieselben Gesichtspunkte ergeben sich auch für

Reptilien, Vögel und Säuger, und sie gelten auch ebenso gut bei allen Vertebraten für die hintere Extremität — es handelt sich um ein festes, die ganze Vertebraten-Reihe beherrschendes Gesetz, dass der Anstoss zur Entwicklung des Gliedmassenskeletes stets von der Peripherie ausgeht, und dass sich die centralen (Gürtel-) Theile erst secundär unter dem formativen Einfluss der freien Gliedmasse entwickeln."

One of course feels some regret that there is not a sufficient number of examples of backward homöosis for the basing of a conclusion in regard to the association of backward homöosis with trunk elongation. The only specimen which shows this unusual homöotic tendency is No. 62, and on a reference to Plate C it will be seen that its XIX intervertebral septum is located on the mean abscissa of 33%.

SECTION VI.

Are variations in the relative position of the pectoral arch associated with variations in the relative position of the pelvic arch?

The pectoral arch, located near the anterior end of the body and closely associated with the head and visceral skeleton, would be expected *a priori* to show a lesser amplitude of variation in respect to the segments of the vertebral axis than is shown by the pelvic arch. In front of it are only three vertebræ, and the limits of vertebral elasticity, if I may use the term, are correspondingly restricted.

On the other hand, the shoulder girdle is not articulated to any one vertebra, and those intimate relations between bones which we have been taught are so potent in determining their position and character, do not here exist, and were it not for the tensile influence of the myotomes the enclosed vertebræ might slide back and forth through the arch *ad libitum*.

Since the girdle thus lies imbedded only in muscle tissue, its normal position in relation to the embraced vertebræ is rather difficult to determine from specimens which have been killed without special care. My observations, however, convince me that the halves of the arch do not always occupy the same position with regard to the vertebral segments.

The small ossified scapulæ are often very clearly shown on both the lateral and dorsal radiographs, and it is not a difficult matter to get an approximate idea of their relation to the neighboring vertebræ. They ordinarily lie opposite the middle of the fourth vertebra, though they may lie opposite the intervertebral space which separates the third vertebra from the fourth, and less frequently opposite the space which separates the fourth from the fifth. They are represented on Plate C by the small cross-lines occurring between abscissas three and four. Let us examine the extreme cases, and see if there is a correlation between pelvic and pectoral variations.

Specimens 12, 28, 30, 31, 35, 38, 39, 43, 52, 73*, 79*, 92, 94* all show the scapulæ to lie opposite or near the third intervertebral space, while specimens 23, 24*, 37*, 53*, 58*, 68*, 71, 77, 78*, 83*, 89*, 90, 95*, 98 show a tendency towards the approach of the fourth intervertebral space.

Now of the first series of thirteen specimens there are only three cases (indicated by an *) of forward homœosis of the sacrum. In the second series, however, of fourteen specimens, there are nine cases of forward homœosis.

When one stops to consider that the total number of homœotic specimens is approximately only one-half the number of normal specimens, and that the chances of scapular variation are hence twice as liable to gather around normal individuals, the figures in the previous paragraph have an increased significance.

We can conclude that variations in the relative position of the pectoral arch *are* associated with variations in the relative position of the pelvic arch, and that both variations are causally connected with some common factor. I believe that factor to be the variation of the relative length of different vertebral regions, as considered in the fifth section.

SECTION VII.

Are there other skeletal variations associated with pelvic variation?

A critical examination of the skeletons of *Necturus*, made for the purpose of detecting a possible tendency on the part of

homœotic individuals towards variations in other directions, leads to the conclusion that homœosis is only an index of general instability on the part of the skeleton as a whole.

(1) This principle was illustrated in Section VI, where it was shown that variations in the position of the sacral vertebra are accompanied by variations, in a definite direction, in the pectoral region.

(2) The principle was also illustrated in Section I, when it was shown that homœotic specimens tend towards numerical increase in the number of vertebral segments. — Before passing on to other possible illustrations, let us examine this present phenomenon more in detail.

According to Bateson's law, forward homœosis, involving one vertebra, should yield a column of *one* more than the normal number of vertebræ. But if we examine the curve of lengths in terms of vertebræ, on Plate C (the lower of the curves drawn in black), we shall find that whereas the normal specimens average only 45 vertebræ, the homœotic average not *one* more, 46, but *two* and *three* more, 47 and 48, and that the amplitude of variation, moreover, is much greater among the latter than among the normal specimens. It is clear, then, that there is an added increase in the variation of the vertebræ among homœotic specimens.

(3) A third illustration of the principle of general variability was given in Section V, where it was shown that abnormalities in the relative lengths of the anterior and posterior portions of the body tend to gather about homœotic individuals.

(4) Dr. Parker ('96) has made use of the first hæmal arch in his comparisons of the vertebral columns of *Necturus*, showing that its position is subject to considerable variation. Let us see if homœotic specimens present greater or less variation in the position of this arch than do the normal examples.

Of sixty-three normal specimens giving satisfactory radiographs of the first hæmal arch,

10 examples (16%)	have the arch attached to the XXII vertebra.
51 " (81%)	" " " " " " " " XXIII "
2 " (3%)	" " " " " " " " XXIV "

and of thirty-five homœotic specimens,

1	example	(3%)	has	the	arch	attached	to	the	XXII	vertebra.
31	examples	(88%)	have	"	"	"	"	"	XXIII	
3	"	(9%)	"	"	"	"	"	"	XXIV	

Our figures show that in both normal and homœotic specimens the point of constancy is the XXIII vertebra, and, moreover, that the homœotic specimens are slightly less liable to vary than the normal, the former presenting 88% of cases of stability against 81% presented by the normal specimens.

It would seem that the production of the first hæmal arch is then a function of the XXIII vertebra, a function of the axial portion of the embryo, and that the development of the hæmal arch proceeds entirely independent of the position of the neighboring appendages, for in 81% of the normal specimens there are three intervening vertebræ between the sacrum and the first hæmal arch, while in nearly all of the homœotic specimens there are but two.

Of course if we assume that the normal position of the first hæmal arch is on the fourth vertebra behind the sacrum, we can then show that the homœotic specimens present a greater range of variation than normal individuals. But this assumption would be unjustified.

(5) Specimens may frequently be found whose caudal vertebræ are provided with abnormal processes. These abnormalities may appear as bi-lobed or double neural plates, or double neural spines. Similar malformations may affect the hæmal arch (Plate B, Spec. 79).

If our belief in the general variability of the skeleton is to be justified, these minor variations should be of more frequent occurrence among the homœotic than among the normal specimens.

Among thirty-six homœotic specimens there are twenty which present vertebral variations in the caudal region, while of sixty-four normal specimens there are only twenty-six that show the same local variability.

It should not be forgotten that imperfect processes of regeneration may be responsible for the large number of caudal malformations, though this does not explain why homœotic individuals should be more frequently captured during the regenerative process.

I wish that alcoholic "mud-puppies" presented some striking coloration, like the spots of *Diemyctylus* or *Amblystoma*, so that one might detect any tendency towards color variation in specimens presenting skeletal instability; few animals, however, are more alike externally than preserved specimens of *Necturus*.

SECTION VIII.

The question may arise: Are variations in the position of the pelvis correlated with sex, or, if not directly dependent upon the sex, does one sex show a greater tendency towards skeletal variation than the other?

It is frequently the case when a large number of individuals of a single species are collected that the males predominate, but in the present collection, as will be seen by counting the indices of sex at the top of Plate C, there are thirty-seven males and sixty-three females. There should be, then, if variation is equally distributed among males and females, nearly twice as many abnormally placed pelvises among the females as among the males. In fact, however, of the thirty-six homœotic specimens, only nine occur among the males, while twenty-seven, three times as many, occur among the females.

It is, I think, quite generally believed that males are much more subject to variation than are females. Montgomery ('96) in his recent paper on "Organic Variation" states that "the dimensions of birds are more variable in the males than in the females," and that "it is not impossible that in birds, as in man, the female may be more conservative and less progressive than the male." This of course recalls Brooks ('83) and Geddes and Thomson ('90), though we should not forget that males are frequently larger among birds and mammals, and thus offer an increased amplitude of variation, and that males are, moreover, frequently provided with many and variable secondary sexual characters whose development is directly dependent upon the activity of the proper sexual glands, and are hence subject to considerable variation.

Among Amphibia, however, it has been many times shown that the male may assume certain of the duties which are

generally considered to be attributes of the female, and it is barely possible that this affectation by the male is only an expression in habit of a general condition of physical conservatism.

SECTION IX.

Are there anatomical grounds for the theory of vertebral intercalation?

If we accept the theory that there is a fixed intimate interrelation between the appendage, girdle, and some one supporting vertebra; that all are parts of one developing area, parts bound together by some intangible law of correlation, the occurrence of the XX vertebra as the support of the pelvic appendages can be explained only by the theory of vertebral intercalation of Albrecht and Baur.

If I understand the theory correctly, it is the function of one particular vertebra — or where more than one vertebra enters into the formation of the sacrum, of certain particular vertebræ — to give attachment to the pelvic arch, and no matter what secondary alterations may occur before or behind, this vertebra or these vertebræ will tenaciously hold to their prescribed and inherited function.

After examining the descriptions of several examples of intercalation given by Baur, in an earlier number of this journal ('91), I fail to see that they necessarily support his theory.

In the first place, Albrecht and Fürbringer are acknowledged to differ in their interpretation of the Belgian python, and the mention of vertebral and costal asymmetry in *Pelamis* and *Cimoliasaurus* does not render the intercalation theory more probable.

The case of the gavial, described by Baur and considered by Parker to show "very conclusively that, in place of one vertebra, two or parts of two may arise," is equally inconclusive. Baur writes: "It is well known that the typical number of the pre-sacral vertebræ in the living Crocodilia is twenty-four; there are two sacrals; the first caudal is peculiar by being convex. In a specimen of *Gavialis gangeticus* I found twenty-five pre-sacral vertebræ. As in all living crocodiles, the first caudal

vertebra is bi-convex; but in this case it is the twenty-eighth, in the other the twenty-seventh. Is it not evident, therefore, that at some place between the occipital condyle and the first caudal a new vertebra has been inserted? By careful comparison I find that this new vertebra has been intercalated between the ninth and tenth."

Dr. Baur does not tell us how he was able to pronounce this an intercalated segment, leaving it to be presumed, however, that a new vertebra possesses certain diagnostic features. Moreover, if perfectly formed sacral ribs may appear through discontinuous variation on the XX vertebra of the "mud-puppy," I see no reason why the new first caudal of the abnormal gavia should not produce a bi-convex rather than a procœlous centrum.

If we could only have the relative trunk and tail measurements of these specimens with abnormal vertebræ, and could compare them with the normal, we could soon tell whether a strange vertebra had been wedged into the regular series, making the trunk abnormally long, or whether the arrest in the development of a trunk vertebra had not resulted in the abortion of certain anterior vertebræ, drawing into the sacral region certain primarily caudal elements.

The *Helodermas* mentioned by Baur, on page 335, would form excellent material for such a comparative study, since the four specimens exhibit four variations in the numerical position of the first caudal vertebra. But in the absence of a large number of *Helodermas*, let us examine again our *Necturus* material and see if we cannot find vertebræ that might be looked upon as intercalated.

Specimen No. 46 shows a slight reduction in the lengths of the fifth and sixth vertebræ, and specimen No. 61 shows a similar reduction in the twelfth and thirteenth. In both cases, the posterior of the affected vertebræ is slightly smaller than the anterior.

If during the process of embryonic growth there was a disturbance of the regular formation of the body segments, we can easily see that this disturbance might have resulted in the interruption of the growth of certain pre-sacral vertebræ. The

general proportions of the respective vertebral regions would thus be altered, and a tendency would result for the XIX segment to be laid down in a relatively more anterior position. The sacrum, in other words, would tend towards forward homœosis. The two specimens just mentioned should, then, present forward homœosis, and both do.

On Baur's ground, also, both specimens should show forward homœosis, but on his ground that portion of the body lying in front of the normal XIX segment should show, in addition, an increase in absolute length proportional to the numerical increase of the vertebræ. In the specimens at hand such a proportional increase in the length of the trunk region does not take place. Specimen No. 46, though possessed of an additional pre-sacral vertebra, has its sacrum, so far as the general proportions of the body are concerned, in the normal position, *i.e.*, on abscissa 33%, as will be observed by reference to Plate C; while No. 61, far from having a longer pre-sacral region, has this region remarkably short; indeed, in the one hundred specimens of which proportional measurements have been taken, only two show an equal amount of pre-sacral compression.

Again, according to the theory of intercalation, if the first hæmal arch occurs normally on the XXIII vertebra, four joints behind the sacrum, on the occasion of the introduction of an additional pre-sacral vertebra, the pelvis should be simply forced backward with all its chattels. This, however, does not occur,—the hæmal arch does not change its position from the XXIII vertebra.

On the theory of regional compression and expansion, which we have advanced, the hæmal arch, a function of the axial rather than of the appendicular areas, should tend in forwardly homœotic specimens to approach the pelvic arch, *i.e.*, in homœotic specimens the number of vertebræ intervening between the sacrum and the first hæmal arch should tend towards reduction. This is what actually occurs. (See Section VII, p. 473.)

The assumption that the embryonic pelvic girdle travels backward and forward over a fixed vertebral column has been

questioned both by Bateson and Parker, though believed by Rosenberg and Credner. With Parker one can agree that the "sacral region has the power of developing sacral ribs at several points on both right and left sides." But Parker gives us no reason for the abnormal position often taken by certain sacral ribs, and does not explain why a particular vertebra (or vertebræ) produces sacral ribs, and why others, potentially able to produce sacral ribs, produce ribs of the ordinary sort. If several vertebræ are endowed with this power, why do we never find nicely formed sacral ribs passing out into the surrounding tissue as if searching for some ilia with which they might articulate, and why are the ilia not occasionally attached to ribs of the ordinary triangular type?

The sacrum, pelvis, and appendages are not intimately associated parts of the body that represent one complete whole, and the definitive location of the sacrum is probably due to centripetal influence derived from the budding appendage. Interpolation in the sense of the introduction of new segments does not take place, and what have been given as examples of interpolation are probably only imperfectly formed body segments.

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Nov. 3, 1896.

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EXPLANATION OF PLATE A.

The figures are printed *directly* from the original radiographs, and, so far as size and general structure are concerned, are faithful reproductions; but the exquisite details and sharp lines of the originals are unfortunately obscured.

SPECIMEN 62 is peculiar in that the pelvis is oblique. The figure also shows the location of the scapulæ. The animal was placed in a supine position upon the photographic plate.

SPECIMEN 62*, the same animal seen from the side.

SPECIMEN 87. This figure shows a symmetrical pelvis, and indicates the position of the scapulæ.



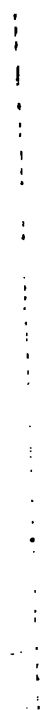
specimen No 62.



*specimen No 62**



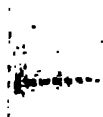
specimen No 62



EXPLANATION OF PLATE B.

SPECIMENS 1 and 2, the smallest in the series, gave, on the original photographic plate, the total number of vertebrae with remarkable clearness. They have greatly suffered in the process of reproduction.

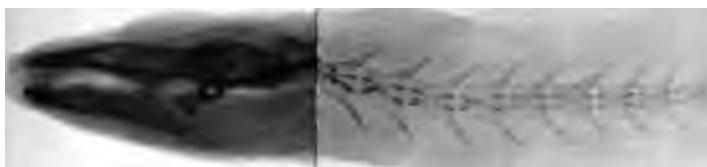
SPECIMEN 79 is especially interesting in that certain of the caudal vertebrae are provided with double neural and haemal spines.



specimen No. 1



specimen No. 2



specimen No. 79



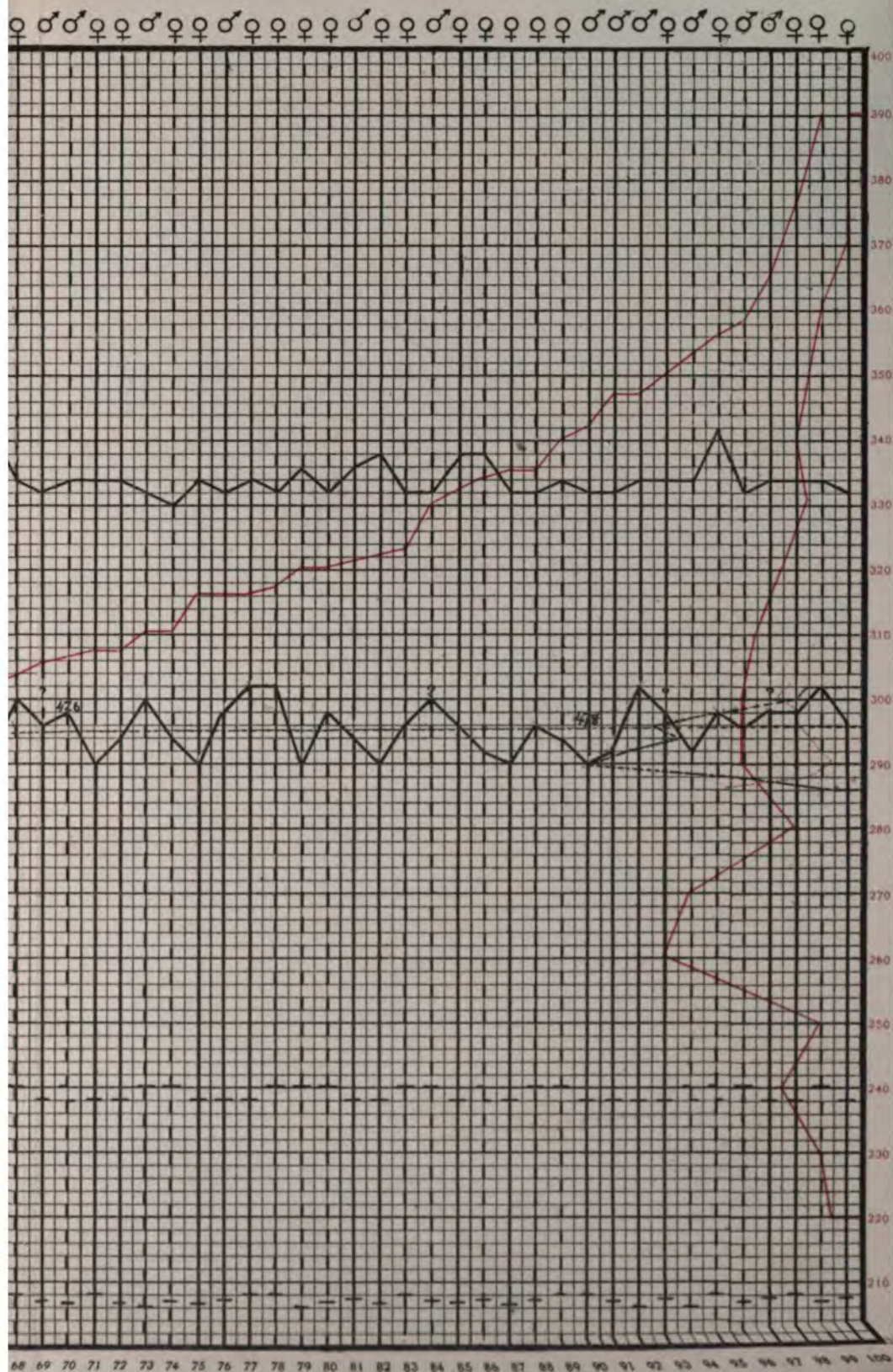
EXPLANATION OF PLATE C.

The vertical lines, ordinates, indicate the several specimens from 1 to 100. Entire lines represent normal, broken lines represent homœotic specimens. The numbers at the left, in ascending order from 1 to 55, indicate the number of vertebrae and the ratio of the length from the XXX to the XX, and XIX to the I vertebra.

The irregular line, crossing the plate transversely from 45 on the left, represents the length of the animals in terms of vertebrae, and the more regular dotted line follows its mean course.

The irregular line extending across the upper part of the plate represents the relative variation in the ratio of tail-length to body-length.

The red curve, running obliquely across the plate, indicates the variation in absolute length, and is represented in profile on the right. The figures 100 and 100 indicate millimeters; thus, specimens 71 and 72 are 3.8 mm. in length.









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